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(71) Applicant *(for all designated States except US)*: EAST CAROLINA UNIVERSITY [US/US]; 210 Spilman Hall, Greenville, NC 27858–4353 (US).

(71)(72) Applicant and Inventor: NYCE, Jonathan, W. [–/US]; 59 Sayre Drive, Princeton, NJ 08540 (US).

(74) Agent: AMZEL, Viviana; Arter & Hadden LLP, Citicorp Plaza, Suite 3400, 725 S. Figueroa Street, Los Angeles, CA 90017–5434 (US).

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(57) Abstract

An in vivo method of selectively delivering a nucleic acid to a target gene or mRNA, comprises the topical administration, e.g. to the respiratory system, of a subject of a therapeutic amount of an oligonucleotide (oligo) that is anti–sense to the initiation codon region, the coding region, the 5' or 3' inton–exon junctions or regions within 2 to 10 nucleotides of the junctions of the gene or antisense to a mRNA complementary to the gene in an amount effective to reach the target polynucleotide and reducing or inhibiting expression. In addition a method of treating and adenosine mediated effect, comprises topically administering to a subject an anti–sense oligo in an amount effective to treat the respiratory, pulmonary, or airway disease. In order to minimize triggering adenosine receptors by their metabolism, the administered oligos have a low content of or are essentially free of adenosine. A pharmaceutical composition and formulations comprise the oligo anti–sense to an adenosine receptor, genes and mRNAs encoding them, genomic and mRNA flanking regions, intron and exon borders and all regulatory and functionally related segments of the genes and mRNAs encoding the polypeptides, their salts and mixtures. Various formulations contain a requisite carrier, and optionally other additives and biologically active agents. The low adenosine or adenosine free (des–A) agent for practicing the method of the invention may be prepared by selecting a target gene(s), genomic flanking region(s), RNA(s) and/or polypeptide(s) associated with a disease(s) or condition(s) afflicting lung airways, obtaining the sequence of the mRNA(s) corresponding to the target gene(s) and/or genomic flanking region(s), and/or RNAs encoding the target polypeptide(s), selecting at least one segment of the mRNA which may be up to 60 % free of thymidine (T) and synthesizing one or more anti–sense oligonucleotide(s) to the mRNA segments which are free of adenosine (A) by substituting a universal base for A when present in the oligonucleotide. The agent may be prepared by selection of target nucleic acid sequences with GC running stretches, which have low T content, and by optionally replacing A in the anti–sense oligonucleotides with a "Universal or alternative base". The agent, composition and formulations are used for prophylactic, preventive and therapeutic treatment of ailments associated with impaired respiration, lung allergy(ies) and/or inflammation and depletion lung surfactant or surfactant hypoproduction, such as pulmonary vasoconstriction, inflammation, allergies, allergic rhynitis, asthma, impeded respiration, lung pain, cystic fibrosis, bronchoconstriction. The present treatment is suitable for administration in combination with other treatments, e.g. before, during and after other treatments, including radiation, chemotherapy, antibody therapy and surgery, among others. Alternatively, the present agent is effectively administered prophylactically or therapeutically by itself for conditions without known therapies or as a substitute for therapies exhibiting undesirable side effects. The treatment of this invention may be administered directly into the respiratory system of a subject so that the agent has direct access to the lungs, or by other effective routes of administration, e.g. topically, transdermally, by implantation, etc., in an amount effective to reduce or inhibit the symptoms of the ailment.

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LOW ADENOSINE ANTI-SENSE OLIGONUCLEOTIDE, COMPOSITIONS, KIT & METHOD FOR TREATMENT OF AIRWAY DISORDERS ASSOCIATED WITH BRONCHOCONSTRICTION, LUNG INFLAMMATION, ALLERGY(IES) & SURFACTANT DEPLETION

BACKGROUND OF THE INVENTION

Field of the Invention

This patent relates to a composition comprising oligonucleotides (oligos) that are anti-sense to adenosine receptors, and contain low amounts of or no adenosine (A). These agents are suitable for the treatment, among others, of pulmonary diseases associated with inflammation, impaired airways, including lung disease and diseases whose secondary effects afflict the lungs of a subject. Examples of these diseases are allergies, asthma, impeded respiration, allergic rhynitis, pain, cystic fibrosis, and cancers such as leukemias, e.g. colon cancer, and the like. The present agent may be administered prophylactically or therapeutically in conjunction with other therapies, or may be utilized as a substitute for therapies that have significant, negative side effects.

Background of the Invention

Respiratory ailments, associated with a variety of diseases and conditions, are extremely common in the general population, and more so in certain ethnic groups, such as African Americans. In some cases they are accompanied by inflammation, which aggravates the condition of the lungs. Asthma, for example, is one of the most common diseases in industrialized countries. In the United States it accounts for about 1% of all health care costs. An alarming increase in both the prevalence and mortality of asthma over the past decade has been reported, and asthma is predicted to be the preeminent occupational lung disease in the next decade. While the increasing mortality of asthma in industrialized countries could be attributable to the depletion reliance upon beta agonists in the treatment of this disease, the underlying causes of asthma remain poorly understood.

Adenosine may constitute an important mediator in the lung for various diseases, including bronchial asthma. Its potential role was suggested by the finding that asthmatics respond favorably to aerosolized adenosine with marked bronchoconstriction whereas normal individuals do not. An asthmatic rabbit animal model, the dust mite allergic rabbit model for human asthma, responded in a similar fashion to aerosolized adenosine with marked bronchoconstriction whereas non-asthmatic rabbits showed no response. More recent work with this animal model suggested that adenosine-induced bronchoconstriction and bronchial hyperresponsiveness in asthma may be mediated primarily through the stimulation of adenosine receptors. Adenosine has also been shown to cause adverse effects, including death, when administered therapeutically for other diseases and conditions in subjects with previously undiagnosed hyper reactive airways.

A handful of medicaments have been available for the treatment of respiratory diseases and conditions, although in general they all have limitations. Theophylline, an important drug in the treatment of asthma, is a known adenosine receptor antagonist which was reported to eliminate adenosine-mediated bronchoconstriction in asthmatic rabbits. A selective adenosine A_1 receptor antagonist, 8-cyclopentyl-1, 3-dipropylxanthine (DPCPX) was also reported to inhibit adenosine-mediated bronchoconstriction and bronchial hyperresponsiveness in allergic rabbits. The therapeutic and preventative applications of currently available adenosine A_1 receptor-specific antagonists are, nevertheless, limited by their toxicity. Theophylline, for example, has been widely used in the treatment of asthma, but is associated with frequent, significant toxicity resulting from its narrow therapeutic dose range. DPCPX is far too toxic to be useful clinically. The fact that, despite decades of extensive research, no specific adenosine receptor antagonist is available for clinical use attests to the general toxicity of these agents. Anti-sense oligonucleotides have received considerable theoretical consideration as potential useful pharmacological agents in human disease. Their practical application in actual models of human disease, however, has been somewhat elusive. One important impediment to their effective application has been a difficulty in finding an appropriate route of administration to

deliver them to their site of action. Many in vivo experiments were conducted by administering anti-sense oligonucleotides directly to specific regions of the brain. These applications, however, necessarily have limited clinical utility due to their invasive nature. Although anti-sense oligonucleotides have received considerable theoretical consideration for their potential use as pharmacological agents in human disease, finding practical and effective applications for these agents in actual models of human disease, however, have been few and far between, particularly because they had to be administered in large doses. Another important consideration in the pharmacologic application of these molecules is their route of administration. Many in vivo applications have involved the direct administration of anti-sense oligonucleotides to limited regions of the brain. Such applications, however, have limited clinical utility due to their invasive nature. The systemic administration of anti-sense oligonucleotides as pharmacological agents has been found to have also significant problems, not the least of which being an inherent difficulty in targeting disease-involved tissues. That is, the necessary dilution of the anti-sense oligonucleotide in the circulatory system makes extremely difficult to attain a therapeutic dose at the target tissue by intravenous or oral administration. The bioavailability of orally administered anti-sense oligonucleotides is very low, of the order of less than about 5%. Anti-sense oligonucleotides have been used in therapy by many, including the present inventor, who in his previous work successfully treated various diseases and conditions by direct administration of these agents to the lung. In many instances, other workers have had to face the difficulties associated with the delivery of DNA molecules to a desired target. Thus, the route of administration may be of extreme importance for treating generalized diseases and conditions as well as those which are localized. In contrast, up to the present time, the delivery of anti-sense agents to the lung has been relatively undeveloped. As described by the present inventor in more detail below, the lung is an excellent target for the direct administration of anti-sense oligonucleotides and provides a non-invasive and a tissue-specific route.

Clearly, there exist presently no effective therapies for treating these ailments, or at least no therapies which are effective and devoid of significant detrimental side effects. Accordingly, there is still a need for an agent for the treatment of adenosine mediated ailments afflicting the pulmonary and respiratory ailments affecting the lung airways, including respiratory problems, bronchoconstriction, inflammation, allergy(ies), depletion or hyposecretion of surfactant, etc., which is highly effective and sufficiently selective to avoid detrimental side effects produced by other therapies. In addition, there is a definite need for making available a delivery method that will require low amounts of therapeutic agents and will be effective for the rapid and targeted access of tissue genes of mRNAs and the reversal of untoward effects afflicting a subject.

SUMMARY OF THE INVENTION

The present invention generally relates to a pharmaceutical or veterinary composition, comprising an anti-sense oligonucleotide(s) (oligo(s)) which is (are) effective for alleviating bronchoconstriction and/or lung inflammation, allergy(ies), and\or surfactant depletion and\or hyposecretion, when administered to a mammal, the oligo containing about 0 to about 15% adenosine (A) and being anti-sense to a target selected from the group consisting of the initiation codon, the coding region, the 5'-end and the 3'-end genomic flanking regions, the 5' and 3' intron-exon junctions, and regions within 2 to 10 nucleotides of the junctions of a gene encoding a target polypeptide associated with lung airway dysfunction or anti-sense to the polypeptide mRNA; combinations of the oligos; and mixtures of the oligos; and a pharmaceutically or veterinarily acceptable carrier or diluent. The targets are typically molecules associated with airway disease, cancer, etc., such as transcription factors, stimulating and activating peptide factors, cytokines, cytokine receptors, chemokines, chemokine receptors, adenosine receptors, bradykinin receptors, endogenously produced specific and non-specific enzymes, immunoglobulins and antibodies, antibody receptors, central nervous system (CNS) and peripheral nervous and non-nervous system receptors, CNS and peripheral nervous and non-nervous system peptide transmitters, adhesion molecules, defensins, growth factors, vasoactive peptides and receptors, binding proteins, and malignancy associated proteins, among others. Examples

are oligo(s) targeted to adenosine receptor(s) and it(they) are typically present in the composition in an amount effective to reduce adenosine mediated effect(s), such as airway obstruction, inflammation, allergy(ies), and sufactant depletion, among others. The adenosine receptor is preferably selected from the group consisting of the adenosine A_1, A_{2b}, and A_3 receptors, and in some instances even adenosine A_{2a} receptors. The oligo of the invention may be applied to the preparation of a medicament for (a) reducing adenosine-mediated bronchoconstriction, impeded respiration, inflammation, allergy(ies), depletion production of surfactant, and other detrimental pulmonary effects in a subject in need of treatment, and/or for (b) treating specific diseases and conditions such as asthma, cystic fibrosis, allergic rhynitis, COPD, etc. For the first time this invention provides the targeted administration of one or more oligonucleotides directly into the repiratory system. The oligos may be directed to any target and are intended for fast delivery through the mucosal tissue of the lungs for hybridization to a desired target polynucleotide, e. g. mRNA, to prevent gene transcription and translation, such that protein expression will be reduced, hampered, or completely stopped. Thus, this invention also provides a more general method for administering oligonucleotides that are anti-sense to targeted genes and mRNAs associated with any type of diseases, by direct administration into the respiratory system, e. g. by inhalation, by introduction of a solution or aerosol into the respiratory airways, and/or directly into the lung.

The present oligos, moreover, are suitable for reducing effects mediated by a variety of target proteins and genes, for example adenosine-mediated effects, including pulmonary, respiratory, and other associated effects, e. g. bronchoconstriction, inflammation, immune mediated reactions, allergy(ies) and other airway problems, which may be caused by different conditions, including cancer. Examples of diseases and conditions, which may be treated preventatively, prophylactically and therapeutically with the agent of this invention, are pulmonary vasoconstriction, inflammation, allergies, asthma, impeded respiration, respiratory distress syndrome, pain, cystic fibrosis, allergic rhynitis, pulmonary hypertension, pulmonary vasoconstriction, emphysema, chronic obstructive pulmonary disease (COPD), bronchitis, and cancers such as leukemias, lymphomas, carcinomas, and the like, e.g. colon cancer, breast cancer, lung cancer, pancreatic cancer, hepatocellular carcinoma, kidney cancer, melanoma, hepatic metastases, etc., as well as all types of cancers which may metastasize or have metastasized to the lung(s), including breast and prostate cancer. The present agents are also suitable for administration before, during and after other treatments, including radiation, chemotherapy, antibody therapy, phototherapy and cancer, and other types of surgery. The present agent is effectively administered prophylactically and therapeutically in conjunction with other therapies, or by itself for conditions without known therapies or as a substitute for therapies that have significant negative side effects. The oligo(s) may be administered by any means known to a subject, e. g. to the lungs of the subject, more generally through any and all systemic and topical routes. This oligonucleotide(s) (oligo(s)) employed are anti-sense to to a target DNA or RNA, e. g. an adenosine receptor DNA or RNA, and preferably consist essentially of up to about 15% adenosine (A), and more preferably contain no adenosine. The oligos are provided in the form of specific compositions and formulations, with a carrier or diluent, and optionally with other therapeutic agents and additives which are used for administration by specific routes, e.g. into the respiratory system, topically, transdermally, parenterally, by implantation, and the like. The oligo is also provided as a capsule or cartridge, and in the form of a kit. The oligos of the invention may be produced by selection of specific targeted segments of the gene or mRNA encoding the adenosine receptor as described below. In one preferred embodiment, the selection is made to obtain oligos that consisting essentially of less than about 15% adenosine (A). This may be done by selecting the target as done above, which includes genes, genomic flanking regions, RNAs and polypeptide associated with an ailment afflicting the lung airways, obtaining the sequence of a mRNA(s) corresponding to the target gene(s) and/or their genomic flanking region(s) and/or the juxta-membrane regions thereof, and mRNA(s) encoding the target polypeptide(s), selecting at least one segment of the mRNA(s), and synthesizing one or more anti-sense oligonucleotide(s) to the selected mRNA segment(s), and substituting, if necessary, an alternative, e. g.

a universal base(s) or other base(s) for one or more A to reduce the proportion of A present in the oligonucleotide to less than about 15%, and down to no adenosine. Similarly, alternative and/or universal bases may be substituted for adenosine, e. g. specific adenosine A1, A2b and A3 receptor antagonists or A2a receptor agonists, theophilline, enprophylline, and many other adenosine receptor antagonists known in the art as well as agonists with significantly reduced agonist activity with respect to adenosine, e. g. less than 0.5%, less than 0.3%, and the like.

The invention will now be described in general in conceptual and experimental terms, with reference to specific examples. Other objects, advantages and features of the present invention will become apparent to those skilled in the art from the description that follows.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

This invention arose from a desire by the inventor to improve on prior art treatments for pulmonary and other diseases, which technology is generally frought with detrimental side effects and by the need of administering high doses of therapeutical agents. The present invention arises from the inventor's own discovery that adenosine receptor targeted anti-sense oligonucleotides (oligos) may be utilized therapeutically in the treatment of diseases or conditions which impair respiration, cause inflammation and/or allergy(ies), constrict bronchial tissue, obstruct the lung airways, depletion surfactant secretion, or otherwise impede normal breathing. In general, many diseases and conditions are associated with or cause inflammation, constrict bronchial tissue or the lung airways, depletion secretion of surfactant, augment allergy(ies), or otherwise impede normal breathing. This treatment is selective for specific targets associated with or mediating these symptoms, and the agents are administered in up to 1000-fold lower doses than those seen in the art. The inventor, in addition, wanted to provide a treatment which would improve the outcome and life style of patients undergoing other procedures or being administered other therapies, including antibody therapy, chemotherapy, radiation, phototherapy, and surgery e.g. cancer surgery, and that could be effectively administered preventatively, prophylactically or therapeutically. He reasoned that he could further improve on this discovery by selecting oligos of reduced adenosine content, or reducing the adenosine content of otherwise targeted anti-sense oligos corresponding to endogenous polynucleotide sequences. The present invention is premised on the discovery by the inventor that oligonucleotides are metabolized in vivo to their mononucleotides. Adenosine (A)-containing oligonucleotides break down and release adenosine which, in turn, activates adenosine receptors, thereby causing bronchoconstriction, inflammation, surfactant depletion, allergy(ies), and the like. He, thus, conceived of employing low adenosine-free adenosine oligos to avoid these side effects upon their administration. He succeeded in this endeavor and is providing in this patent novel and improved compositions, formulations and methods which afford greatly improved results when compared with previously known treatments for preventing and alleviating bronchoconstriction, allergy(ies), inflammation, breathing difficulties, surfactant depletion and blockage of airways, as well as for other conditions which affect the lung directly or indirectly. In different embodiments, one or more nucleic acids of the invention may be formulated alone, and/or with one or more surfactant components and/or with a carrier, and/or with other therapeutic agents and/or formulation agents known in the art. The compositions of this invention, thus, may be incorporated into a variety of formulations for systemic and topical administration. Moreover, the inventor also provides a broad method for delivery of anti-sense oligonucleotides (oligos) through the respiratory system, as a fast means of starting treatment to address acute attacks of asthma and other diseases and conditions that have a rapid onset. In addition, the present agents have long halflives and may be administered at very low doses. This makes them ideal for once a week type therapies. In the past, anti-sense oligonucleotides received considerable theoretical consideration as being potentially useful as pharmacologic agents for the treatment of human disease. Wagner, R., Nature 372: 333-335 (1994). However, it has been difficult to actually apply these molecules to alleviating and curing human diseases. One important consideration in the pharmacologic application of these molecules has been the failure of various routes of administration to deliver the compounds to its target while avoiding invading the circulation and, therefore, other

untargeted tissues which, thus, produces a plethora of side effects. Most in vivo experiments utilizing anti-sense oligonucleotides involved a direct application of the oligo to limited regions of the brain. See, Wahlestedt, C., Trends in Pharmacol. Sci. 15: 42-46 (1994); Lai, J. et al., Neuroreport 5: 1049-1052 (1994); Standifer, K., et al., Neuron 12: 805-810 (1994); Akabayashi, A., et al., Brain Res. 21: 55-61 (1994). Others applied them into the spinal fluid. See, e.g. Tseng, L., et al., European J. Pharmacol. 258: R1-3 (1994); Raffa, R., et al., European J. Pharmacol. 258: R5-7 (1994); Gillardon, F., et al., European J. Neurosci. 6: 880-884 (1994). Such applications, clearly, have no practical clinical utility due to their invasive nature. Thus, the systemic administration of anti-sense oligonucleotides poses significant problems with respect to their pharmacologic application, not the least of which is the difficulty in selectively targeting disease-involved tissues. The systemic administration of anti-sense oligonucleotides also poses significant problems with respect to their pharmacologic application, not the least of which is the difficulty in selectively targeting disease-involved tissues.

The respiratory system, and in particular the lung, as the ultimate port of entry into the organism, however, is an excellent route of administration for anti-sense oligonucleotides. This is so not only for the treatment of lung disease, but also when utilizing the lung as a means for delivery, particularly because of its non-invasive and tissue-specific nature. Thus, local delivery of antisense oligonucleotides directly to the target tissue enables the therapeutic use of these compounds. Fomivirsen (ISIS 2302) is an example of a local drug delivery into the eye to treat cytomegalovirus (CMV) retinitis, for which a new drug application has been filed by ISIS. The administration of a drug through the lung offers the further advantage that inhalation is non-invasive whereas direct injection in to the vitreous of the eye is invasive. The composition and formulations of this invention are highly efficacious for preventing and treating diseases and conditions associated with bronchoconstriction, difficult breathing, impeded and obstructed lung airways, allergy(ies), inflammation and surfactant depletion, among others. Examples of diseases and conditions which are suitably treated by the present method are diseases and conditions, including Acute Respiratory Distress Syndrome (ARDS), asthma, adenosine administration e.g. in the treatment of SupraVentricular Tachycardia (SVT) and other arrhythmias, and in stress tests to hyper-sensitized individuals, ischemia, renal damage or failure induced by certain drugs, infantile respiratory distress syndrome, pain, cystic fibrosis, pulmonary hypertension, pulmonary vasoconstriction, emphysema, chronic obstructive pulmonary disease (COPD), lung transplantation rejection, pulmonary infections, and cancers such as leukemias, lymphomas, carcinomas, and the like, including colon cancer, breast cancer, lung cancer, pancreatic cancer, hepatocellular carcinoma, kidney cancer, melanoma, hepatic metastases, etc., as well as all types of cancers which may metastasize or have metastasized to the lung(s), including breast and prostate cancer. The invention will be described with respect to the adenosine receptors as targets, but is similarly applicable to any other target with respect to the pulmonary administration of anti-sense oligos. The examples provided below show a complete inhibition of such adenosine receptor associated symptoms in a rabbit model for human bronchoconstriction, allergy(ies) and inflammation as well as the elimination of the ability of the adenosine receptor agonist par excellence, adenosine, to cause bronchoconstriction in hyper-responsive monkeys, which are animal models for human hyper-responsiveness to adenosine receptor agonists. The pharmaceutical composition and formulations of the invention, therefore, are suitable for preventing and alleviating the symptoms associated with stimulation of adenosine receptors, such as the adenosine A_1 receptors. The compositions and formulations of this invention, thus, are also suitable for prevent the untoward side effects of adenosine-mediated hyperresponsiveness in certain individuals, which are generally seen in diseases affecting respiratory activity.

The method of the present invention may be used to treat airway diseases and conditions in a subject of any kind and for any reason, with the intention that the adenosine content of anti-sense compounds be minimized, reduced or eliminated so as to prevent its liberation upon anti-sense degradation. Examples of diseases and conditions, which may be treated preventatively,

prophylactically and therapeutically with the compositions and formulations of this invention, are pulmonary vasoconstriction, inflammation, allergies, asthma, allergic rhynitis, impeded respiration, Acute Respiratory Distress Syndrome (ARDS), renal damage and failure associated with ischemia as well as the administration of certain drugs, side effects associated with adenosine administration e.g. in SupraVentricular Tachycardia (SVT) and in adenosine stress tests, infantile Respiratory Distress Syndrome (infantile RDS), ARDS, pain, cystic fibrosis, pulmonary hypertension, pulmonary vasoconstriction, emphysema, chronic obstructive pulmonary disease (COPD), lung transplantation rjejection, pulmonary infections, and cancers such as leukemias, lymphomas, carcinomas, and the like, e.g. colon cancer, breast cancer, lung cancer, pancreatic cancer, hepatocellular carcinoma, kidney cancer, melanoma, metastatic cancer such as hepatic metastases, lung, breast and prostate metastases, among others. The present compositions and formulations are suitable for administration before, during and after other treatments, including radiation, chemotherapy, antibody therapy, phototherapy and cancer, and other types of surgery. The present compositions and formulations may also be administered effectively as a substitute for therapies that have significant negative side effects.The terms "anti-sense" oligonucleotides generally refers to small, synthetic oligonucleotides, resembling single-stranded DNA, which in this patent are applied to the inhibition of gene expression by inhibition of a target messenger RNA (mRNA). See, Milligan, J. F. et al., J. Med. Chem. 36(14), 1923-1937 (1993), the relevant portion of which is hereby incorporated in its entirety by reference. For consistency=s sake, all RNAs and oligonucleotides are represented in this patent by a single strand in the 5' to 3' direction, when read from left to right, although their complementary sequence(s) is (are) also encompassed within the four corners of the invention. In addition, all nucleotide bases and amino acids are represented utilizing the recommendations of the IUPAC-IUB Biochemical Nomenclature Commission, or by the known 3-letter code (for amino acids). Nucleotide sequences are presented herein by single strand only, in the 5' to 3' direction, from left to right. In addition, nucleotide and amino acids are represented herein in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or (for amino acids) by three letter code, in accordance with 37 CFR ' 1.822 and established usage. See, e.g., PatentIn User Manual, 99-102 (Nov. 1990) (U.S. Patent and Trademark Office, Office of the Assistant Commissioner for Patents, Washington, D.C. 20231); U.S. Patent No. 4,871,670 to Hudson et al. at col. 3, lines 20-43. The present method utilizes anti-sense agents to inhibit or down-regulate gene expression of target genes, including those listed in Tables 1 and 2 below. This is generally attained by hybridization of the anti-sense oligonucleotides to coding (sense) sequences of a targeted messenger RNA (mRNA), as is known in the art. The exogenously administered agents of the invention decrease the levels of mRNA and protein encoded by the target gene and/or cause changes in the growth characteristics or shapes of the thus treated cells. See, Milligan et al. (1993); Helene, C. and Toulme, J. Biochim. Biophys. Acta 1049, 99-125 (1990); Cohen, J. S. D., Ed., Oligodeoxynucleotides as Anti-sense Inhibitors of Gene Expression; CRC Press: Boca Raton, FL (1987), the relevant portion of which is hereby incorporated in its entirety by reference. As used herein, "anti-sense oligonucleotide or asnti-sense oligo" is generally a short sequence of synthetic nucleotide that (1) hybridizes to any segment of a mRNA encoding a targeted protein under appropriate hybridization conditions, and which (2) upon hybridization causes a decrease in gene expression of the targeted protein. The terms "desAdenosine" (desA) and "des-thymidine" (desT) refer to oligonucleotides substantially lacking either adenosine (desA) or thymidine (desT). In some instances, the des A or des T sequences are naturally occurring, and in others they may result from substitution of an undesirable nucleotide (A) by another lacking its undesirable activity, such as acting as an agonist or having a triggering effect at the adenosine A receptor(s). In the present context, the substitution is generally accomplished by substitution of A with a "universal or alternative base", presently known in the art or to be ascertained at a later time. As used herein, the terms "prevent", "preventing", "treat" or "treating" refer to a preventative, prophylactic, maintenance, or therapeutic treatment which decreases the likelihood that the subject administered such treatment will manifest symptoms associated with adenosine receptor stimulation. The term "down-regulate" refers to inducing

a decrease in production, secretion or availability and, thus, a decrease in concentration, of intracellular target product, be it a receptor e. g. adenosine A_1, A_{2b}, A_3, bradykinin 2B, GATA-3, or other receptors, or an increase in concentration of the adenosine A_{2a} receptor. The present technology relies on the design of anti-sense oligos targeted to mRNAs associated with ailments involving lung airway pathology(ies), and on their modification to reduce the occurrence of undesirable side effects caused by their release of adenosine upon breakdown, while preserving their activity and efficacy for their intended purpose. In this manner, the inventor targets a specific gene to design one or more anti-sense oligonucleotide(s) (oligos) that selectively bind(s) to the corresponding mRNA, and then reduces, if necessary, their content of adenosine via substitution with an alternative or a universal base, or an adenosine analog incapable of significantly, or having substantially reduced ability for, activating or antagonizing adenosine A_1, A_{2b} or A_3 receptors or which may act as an agonist at the adenosine A_{2a}, receptor. Any number of adenosines present may be substituted by an alternative and/or universal base, such as heteroaromatic bases, which binds to a thymidine base but has less than about 0.3 of the adenosine base agonist or antagonist activity at the adenosine A_1, A_{2a}, A_{2b} and A_3 receptors. Based on his prior experience in the field, the inventor reasoned that in addition to "downregulating" specific genes, he could increase the effect of the agent(s) administered by either selecting segments of RNA that are devoid, or have a low content, of thymidine (T) or, alternatively, substitute one or more adenosine(s) present in the designed oligonucleotide(s) with other nucleotide bases, so called universal bases, which bind to thymidine but lack the ability to activate adenosine receptors and otherwise exercise the constricting effect of adenosine in the lungs, etc. Given that adenosine (A) is a nucleotide base complementary to thymidine (T), when a T appears in the RNA, the anti-sense oligo will have an A at the same position.

In one aspect of this invention, the anti-sense oligonucleotide has a sequence which specifically binds to a portion or segment of a mRNA molecule which encodes a protein associated with impeded breathing, allergy(ies), lung inflammation, depletion of lung surfactant or lowering of lung surfactant, airway obstruction, bronchitis, and the like. One effect of this binding is to reduce or even prevent the translation of the corresponding mRNA and, thereby, reduce the available amount of target protein in the subject=s lung. In one preferred embodiment of this invention, the phosphodiester residues of the anti-sense oligonucleotide are modified or substituted. Chemical analogs of oligonucleotides with modified or substituted phosphodiester residues, e.g., to the methylphosphonate, the phosphotriester, the phosphorothioate, the phosphorodithioate, or the phosphoramidate, α= methoxy ethyl and similar modifications, which increase the in vivo stability of the oligonucleotide are particularly preferred. The naturally occurring phosphodiester linkages of oligonucleotides are susceptible to some degree of degradation by cellular nucleases. Many of the residues proposed herein, on the contrary, are highly resistant to nuclease degradation. See, Milligan et al.; Cohen, J. S. D., supra. In another preferred embodiment of the invention, the oligonucleotides may be protected from degradation by adding a "3'-end cap" by which nuclease-resistant linkages are substituted for phosphodiester linkages at the 3' end of the oligonucleotide. See, Tidd, D. M. and Warenius, H.M., Be. J. Cancer 60: 343-350 (1989); Shaw, J.P. et al., Nucleic Acids Res. 19: 747-750 (1991), the relevant section of which are incorporated in their entireties herein by reference. Phosphoramidates, phosphorothioates, and methylphosphonate linkages all function adequately in this manner for the purposes of this invention, as do α' modifications, such as α' methoxy ethyl, and the like. The more extensive the modification of the phosphodiester backbone the more stable the resulting agent, and in many instances the higher their RNA affinity and cellular permeation. See, Milligan, et al., supra. In addition, a plurality of substitutions to the carbohydrate ring are also known to improve stability of nucleic acids. Thus, the number of residues which may be modified or substituted will vary depending on the need, target, and route of administration, and may be from 1 to all the residues, to any number in between. Many different methods for replacing the entire phosphodiester backbone with novel linkages are known. See, Millikan et al, supra. Preferred backbone analogue residues include phosphoramidate, phosphorothioate, methylphosphonate, phosphorotriester, phosphotriester, thioformacetal,

phosphorodithioate, phosphoramidate, formacetal, triformacetal, thioether, carbamate, boranophosphate, 3'-thioformacetal, 5'-thioether, carbonate, C_5-substituted nucleotides, 5'-N-carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, 2'-O methyl, sulfoxide, sulfide, hydroxylamine, methylene(methylimino) (MMI), methoxymethyl (MOM), and methoxyethyl(MOE), and methyleneoxy(methylimino) (MOMI) residues, and combinations thereof. Phosphorothioate and methylphosphonate-modified oligonucleotides are particularly preferred due to their availability through automated oligonucleotide synthesis. See, Millikan et al, supra. Where appropriate, the agent of this invention may be administered in the form of their pharmaceutically acceptable salts, or as a mixture of the anti-sense oligonucleotide and its salt. In another embodiment of this invention, a mixture of different anti-sense oligonucleotides or their pharmaceutically acceptable salts is administered. A single agent of this invention has the capacity to attenuate the expression of a target mRNA and/or various agents to enhance or attenuate the activity of a pathway. By means of example, the present method may be practiced by identifying all possible deoxyribonucleotide segments which are low in thymidine (T) or deoxynucleotide segments low in adenosine (A) of about 7 or more mononucleotides, preferably up to about 60 mononucleotides, more preferably about 10 to about 36 mononucleotides, and still more preferably about 12 to about 21 mononucleotides, in a target mRNA or a gene, respectively. This may be attained by searching for mononucleotide segments within a target sequence which are low in, or lack thymidine (RNA), a nucleotide which is complementary to adenosine, or that are low in adenosine (gene), that are 7 or more nucleotides long. In most cases, this search typically results in about 10 to 30 such sequences, i.e. naturally lacking or having less than about 40% adenosine, anti-sense oligonucleotides of varying lengths for a typical target mRNA of average length, i.e., about 1800 nucleotides long. Those with high content of T or A, respectively, may be fixed by substitution of a universal base for one or more As. The agent(s) of this invention may be of any suitable length, including but not limited to, about 7 to about 60 nucleotides long, preferably about 12 to about 45, more preferably up to about 30 nucleotides long, and still more preferably up to about 21, although they may be of other lengths as well, depending on the particular target and the mode of delivery. The agent(s) of the invention may be directed to any and all segments of a target RNA. One preferred group of agent(s) includes those directed to an mRNA region containing a junction between an intron and an exon. Where the agent is directed to an intron/exon junction, it may either entirely overlie the junction or it may be sufficiently close to the junction to inhibit the splicing-out of the intervening exon during processing of precursor mRNA to mature mRNA, e.g. with the 3' or 5' terminus of the anti-sense oligonucleotide being positioned within about, for example, within about 2 to 10, preferably about 3 to 5, nucleotide of the intron/exon junction. Also preferred are anti-sense oligonucleotides which overlap the initiation codon, and those near the 5' and 3' termini of the coding region. The flanking regions of the exons may also be targeted as well as the spliced segments in the precursor mRNAs. The mRNA sequences of the adenosine receptors and of many other targets are derived from the DNA base sequence of the gene expressing either receptors, e. g. the adenosine receptors, the enzymes, factors, or other targets associated with airway disease. For example, the sequence of the genomic human A_1 adenosine receptor is known and is disclosed in U.S. Patent No. 5,320,963 to Stiles, G., et al. The A_3 adenosine receptor has been cloned, sequenced and expressed in rat (see, Zhou, F., et al., P.N.A.S. (USA) 89: 7432 (1992)) and human (see, Jacobson, M. A., et al., U.K. Patent Application No. 9304582.1 (1993)). The sequence of the adenosine A_{2b} receptor gene is also known. See, Salvatore, C. A., Luneau, C. J., Johnson, R. G. and Jacobson, M., Genomics (1995), the relevant portion of which is hereby incorporated in its entirety by reference. The sequences of many of the remaining exemplary target genes are also known. See, GenBank, NIH. The sequences of those genes whose sequences are not yet available may be obtained by isolating the target segments applying technology known in the art. Once the sequence of the gene, its RNA and/or the protein are known, an anti-sense oligonucleotides may be produced according to this invention as described above to reduce the production of the targeted protein in accordance with standard techniques. The sequences for the adenosine A_{2a} bradykinin, and other genes as well as methods for preparation of

oligonucleotides are also known as those of many other target genes and mRNAs for which this invention is suitable. Thus, anti-sense oligonucleotides that downregulate the production of target sequences associated with airway disease, including the adenosine A_1, A_{2a}, A_{2b}, A_3, bradykinin, GATA-3, COX-2, and many other receptors, may be produced in accordance with standard techniques. Examples of diseases and conditions which are suitably treated by the present method are diseases and conditions, including Acute Respiratory Distress Syndrome (ARDS), asthma, adenosine administration e.g. in the treatment of SupraVentricular Tachycardia (SVT) and other arrhythmias, and in stress tests to hyper-sensitized individuals, ischemia, renal damage or failure induced by certain drugs, infantile respiratory distress syndrome, pain, cystic fibrosis, pulmonary hypertension, pulmonary vasoconstriction, emphysema, chronic obstructive pulmonary disease (COPD), pulmonary transplantation rejection, pulmonary infections, and cancers such as leukemias, lymphomas, carcinomas, and the like, including colon cancer, breast cancer, lung cancer, pancreatic cancer, hepatocellular carcinoma, kidney cancer, melanoma, hepatic metastases, etc., as well as all types of cancers which may metastasize or have metastasized to the lung(s), including breast and prostate cancer.

The adenosine receptors discussed above are mere examples of the high power of the inventor=s technology. In fact, a large number of genes may be targeted in a similar manner by the present agent(s), to reduce or down-regulate protein expression. By means of example, if the target disease or condition is one associated with impeded or reduced breathing, bronchoconstriction, chronic bronchitis, pulmonary bronchoconstriction and/or hypertension, chronic obstructive pulmonary disease (COPD), pulmonary transplantation rejection, pulmonary infections, allergy, asthma, cystic fibrosis, respiratory distress syndrome, cancers, which either directly or by metastasis afflict the lung, the present method may be applied to a list of potential target mRNAs, which includes the targets listed in Table 1 and Table 2 below, among others. The anti-sense agent(s) of the invention have a low A content to prevent its liberation upon in vivo degradation of the agent(s). For example, if the system is the pulmonary or respiratory system, a large number of genes is involved in different functions, including those listed in Table 1 below.

Table 1: Pulmonary Disease or Condition Pulmonary and Inflammation Targets

Nf6B Transcription Factor	Interleukin-8 Receptor (IL-8 R)
Interleukin-5 Receptor (IL-5R)	Interleukin-4 Receptor (IL-4R)
Interleukin-3 Receptor (IL-3R)	Interleukin-1β (IL-1β)
Interleukin-1β Receptor (IL-1βR)	Eotaxin
Tryptase	Major Basic Protein
β2-adrenergic Receptor Kinase	Endothelin Receptor A
Endothelin Receptor B	Preproendothelin
Bradykinin B2 Receptor (B2BR)	IgE (High Affinity Receptor)
Interleukin-1 (IL-1)	Interleukin 1 Receptor (IL-1 R)
Interleukin-9 (IL-9)	Interleukin-9 Receptor (IL-9 R)
Interleukin-11 (IL-11)	Interleukin-11 Receptor (IL-11 R)
Inducible Nitric Oxide Synthase	Cyclooxygenase (COX)
Intracellular Adhesion Molecule 1 (ICAM-1)	Vascular Cellular Adhesion Molecule (VCAM)
Substance P	Endothelial Leukocyte Adhesion Molecule Endothelin (ELAM-1)
Rantes	GM-CSF, Endothelin-1
ETA Receptor	Neutrophil Chemotactic Factor
Cyclooxygenase-2 (COX-2)	Defensin 1,2,3
Monocyte Activating Factor	Platelet Activating Factor
Neutrophil Elastase	5-lipoxygenase
Muscarinic Acetylcholine Receptors	Substance P
Tumor Necrosis Factor α	Histamine Receptor
Phosphodiesterase IV	CCR-1 CC Chemokine Receptor
Substance P Receptor	Interleukin-4 (IL-4)
Chymase	
Interleukin-2 (IL-2)	

Interleukin-12 (IL-12)	Interleukin-5 (IL-5)
Interleukin-6 (IL-6)	Interleukin-7 (IL-7)
Interleukin-8 (IL-8)	Interleukin-12 Receptor (IL-12R)
Interleukin-7 Receptor (IL-7R)	Interleukin-1 (IL-1)
Interleukin-14 Receptor (IL-14R)	Interleukin-14
CCR-2 CC Chemokine Receptor	CCR-3 CC Chemokine Receptor
CCR-4 CC Chemokine Receptor	CCR-5 CC Chemokine Receptor
Prostanoid Receptors	GATA-3 Transcription Factor
Neutrophil Adherence Receptor	MAP Kinase
Interleukin-15 (IL-15)	Interleukin-15 Receptor (IL-15R)
Interleukin-11 (IL-11)	Interleukin-11 Receptor (IL-11R)
NFAT Transcription Factors	STAT 4
MIP-1α	MCP-2
MCP-3	MCP-4
Cyclophillin (A, B, etc.)	Phospholipase A2
Basic Fibroblast Growth Factor	Metalloproteinase
CSBP/p38 MAP Kinase	Tryptase Receptor
PDG2	Interleukin-3 (IL-3)
Interleukin-10 (IL-10)	Cyclosporin A - Binding Protein
FK506-Binding Protein	α4β1 Selectin
Fibronectin	α4β7 Selectin

Table 1: Pulmonary Disease or Condition Pulmonary and Inflammation Targets

cMad CAM-1	LFA-1 (CD11a/CD18)
PECAM-1	LFA-1 Selectin
C3bi	PSGL-1
E-Selectin	P-Selectin
CD-34	L-Selectin
p150,95	Mac-1 (CD11b/CD18)
Fucosyl transferase	VLA-4
STAT-1	STAT-2
CD-18/CD11a	CD11b/CD18
ICAM2 and ICAM3	C5a
CCR3 (Eotaxin Receptor)	CCR1, CCR2, CCR4, CCR5
LTB-4	AP-1 Transcription Factor
Protein kinase C	Cysteinyl Leukotriene Receptor
Tachykinnen Receptors (tach R)	I6B Kinase 1 & 2
Interleukin-2 Receptor (IL-2R)	(e.g., Substance P, NK-1 & NK-3 Receptors)
STAT 6	c-mas
NF-Interleukin-6 (NF-IL-6)	Interleukin-10 Receptor (IL-10R)
Interleukin-3 (IL-3)	Interleukin-2 Receptor (IL-2R)
Interleukin-13 (IL-13)	Interleukin-12 Receptor (IL-12R)
Interleukin-14 (IL-14)	Interleukin-6 Receptor (IL-6R)
Interleukin-16 (IL-16)	Interleukin-13 Receptor (IL-13R)
Medullasin	Interleukin-16 Receptor (IL-16R)
Adenosine A_1 Receptor (A_1 R)	Tryptase-I
Adenosine A_{2b} Receptor (A_{2b} R)	Adenosine A_3 Receptor (A_3 R)
β Tryptase	STAT-3
Adenosine A_{2a} Receptor (A_{2a} R)	IgE Receptor β Subunit (IgE R β)
Fc-epsilon receptor CD23 antigen	IgE Receptor α Subunit (IgE R α)
IgE Receptor Fc Epsilon Receptor (IgERFc ξ R)	Substance P Receptor
Histidine decarboxylase	Tryptase-1
Prostaglandin D Synthase	Eosinophil Cationic Protein
Eosinophil Derived Neurotoxin	Eosinophil Peroxidase
Endothelial Nitric Oxide Synthase	Endothelial Monocyte Activating Factor
Neutrophil Oxidase Factor	Cathepsin G
Macrophage Inflammatory Protein-1-Alpha/Rantes Receptor	Interleukin-8 Receptor α Subunit (IL-8 Rα)
	Endothelin Receptor ET-B

These genes, and others, are involved in the normal functioning of respiration as well as in diseases associated with respiratory pathologies, including cystic fibrosis, asthma, pulmonary hypertension and vasoconstriction, chronic obstructive pulmonary disease (COPD), pulmonary transplantation rejection, pulmonary infections, chronic bronchitis, respiratory distress syndrome (ARDS), allergic rhinitis, lung cancer and lung metastatic cancers and other airway diseases, including those with inflammatory response.

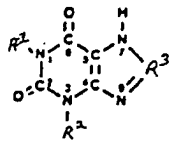
Anti-sense oligos to the target receptors, e. g. the adenosine A_1, A_{2a}, A_{2b}, and A_3 receptors, CCR3 (chemokine receptors), bradykinin 2B, CAM (vascular cell adhesion molecule), and eosinophil receptors, among others, have been shown to be effective in down-regulating the expression of their genes. Some of these act to alleviate the symptoms or reduce respiratory ailments and/or inflammation, for example, by "down regulation" of the adenosine A_1, A_{2a}, A_{2b}, and/or A_3 receptors and CCR3, bradykinin 2B, VCAM (vascular cell adhesion molecule) and eosinophil receptors. These agents may be utilized by the present method alone or in conjunction with anti-sense oligos targeted to other genes to validate pathway and/or networks in which they are involved. For better results, the oligos are preferably administered directly into the respiratory system, e.g., by inhalation or other means, of the experimental animal, so that they may reach the lungs without widespread systemic dissemination. This permits the use of low agent doses as compared with those administered systemically or by other generalized routes and, consequently, reduces the number and degree of undesirable side effects resulting from the agent=s widespread distribution in the body. The agent(s) of this invention has (have) been shown to reduce the amount of receptor protein expressed by the tissue. These agents, thus, rather than merely interacting with their targets, e.g. a receptor, lower the number of target proteins that other drugs may interact with. In this manner, the present agent(s) afford(s) extremely high efficacy with low toxicity. Anti-sense oligonucleotides to the A_1, A_{2b}, A_3, bradykinin B2, GATA-3, CAM (vascular cell adhesion molecule), eosinophil receptors, and COX-2 receptors, among others, have been shown to be effective in the down-regulation of the respective receptor proteins in the cell. One novel feature of this treatment, as compared to traditional treatments for adenosine-mediated bronchoconstriction, is that administration is direct to the lungs, or in situ to other tissues, organs or systems of the body. Additionally, a receptor protein itself is reduced in amount, rather than merely interacting with a drug, and toxicity is reduced. Other proteins that may be targeted with anti-sense agents for the treatment of lung conditions include, but are not limited to: CCR3 (chemokine) receptors, human A_{2a} adenosine receptor, human A_{2b} adenosine receptor, human IgE receptor β, human Fc-epsilon receptor CD23 antigen, human histidine decarboxylase, human beta tryptase, human tryptase-I, human prostaglandin D synthase, human cyclooxigenase-2, human eosinophil cationic protein, human eosinophil derived neurotoxin, human eosinophil peroxidase, human intercellular adhesion molecule-1 (ICAM-1), human vascular cell adhesion molecule-1 (VCAM-1), human endothelial leukocyte adhesion molecule-1 (ELAM-1), human P selectin, human endothelial monocyte activating factor, human IL-3, human IL-4, human IL-5, human IL-6, human IL-8, human monocyte-derived neutrophil chemotactic factor, human neutrophil elastase, human neutrophil oxidase factor, human cathepsin G, human defensin 1, human defensin 3, human macrophage inflammatory protein-1-alpha, human muscarinic acetylcholine receptor HM3, human fibronectin, human GM-CSF, human tumor necrosis factor α, human leukotriene C4 synthase, human major basic protein, and human endothelin 1. Although not intended to be exclusive, a more extensive list of genes is provided below. Some of these act to alleviate the symptoms or reduce respiratory ailments and/or inflammation, for example, by "down regulation" of the adenosine A_1, A_{2a}, A_{2b}, and/or A_3 receptors and CCR3, bradykinin 2B, VCAM (vascular cell adhesion molecule) and eosinophil receptors. These agents are preferably administered directly into the respiratory system, e.g., by inhalation or other means, so that they may reach the lungs without widespread systemic dissemination. This permits the use of substantially lower doses of the agent of the invention as compared with those administered by the prior art, systemically or by other generalized routes and, consequently, reduce undesirable side effects resulting from the agent=s widespread distribution in the body. The agent(s) of this invention has

(have) been shown to reduce the amount of receptor protein expressed by the tissue. These agents, thus, rather than merely interacting with their targets, e.g. a receptor, lower the number of target proteins that other drugs may interact with. In this manner, the present agent(s) afford(s) extremely high efficacy with low toxicity. In these latter targets, and in target genes in general, it is particularly imperative to eliminate or reduce the adenosine content of the corresponding anti-sense oligonucleotide to prevent their breakdown products from liberating adenosine.

As used herein, the term "treat" or "treating" asthma refers to a treatment which decreases the likelihood that the subject administered such treatment will manifest symptoms of the lung disease. The term "downregulate" refers to inducing a decrease in production, secretion or availability (and thus a decrease in concentration) of the targeted intracellular protein. The present invention is concerned primarily with the treatment of human subjects. However, the agents and methods disclosed here may also be employed for veterinary purposes, such as is the case in the treatment of other mammals, such as cattle, horses, wild animals, zoo animals, and domestic animals, e. g. dogs and cats. Targeted proteins are preferably mammalian and more preferably of the same species as the subject being treated. In general, "anti-sense" refers to the use of small, synthetic oligonucleotides, resembling single-stranded DNA, to inhibit gene expression by inhibiting the function of the target messenger RNA (mRNA). Milligan, J. F. et al., J. Med. Chem. 36(14), 1923-1937 (1993). In the present invention, inhibition of gene expression of the A_1 or A_3 adenosine receptor is desired. Gene expression is inhibited through hybridization to coding (sense) sequences in a specific messenger RNA (mRNA) target by hydrogen bonding according to Watson-Crick base pairing rules. The mechanism of anti-sense inhibition is that the exogenously applied oligonucleotides decrease the mRNA and protein levels of the target gene or cause changes in the growth characteristics or shapes of the cells. Id. See, also Helene, C. and Toulme, J., Biochim. Biophys. Acta 1049, 99-125 (1990); Cohen, J. S. D., Ed., Oligodeoxynucleotides as Anti-sense Inhibitors of Gene Expression; CRC Press: Boca Raton, FL (1987). As used herein, "anti-sense oligonucleotide" is defined as a short sequence of synthetic nucleotide that (1) hybridizes to any coding sequence in an mRNA which codes for the targeted protein, according to hybridization conditions described below, and (2) upon hybridization causes a decrease in gene expression of the A_1 or A_3 adenosine receptor. The receptors discussed above are mere examples of the high power of the present technology. In fact, a large number of genes may be targeted in a similar manner by practicing the present methods, to significantly down-regulate or obliterate protein expression and observe any changes wrought to one or more functions within a system, e.g. the respiratory system and other lung disease associated targets. By means of example, in the respiratory system, the targets may be associated with difficulties of breathing, bronchoconstriction, inflammation, allergic rhynitis, chronic bronchitis, surfactant depletion, and others associated with diseases and conditions such as chronic obstructive pulmonary disease (COPD), pulmonary transplantation rejection, pulmonary infections, inhalation burns, Acute Respiratory Distress Syndrome (ARDS), cystic fibrosis, pulmonary fibrosis, radiation pulmonitis, tonsilitis, emphysema, dental pain, oral inflammation, joint pain, esophagitis, cancers afflicting the respiratory system either directly such as lung cancer, esophageal cancer, and the like, or indirectly by means of metastases, among others. These functions are of great interest because of their association with respiratory dysfunction, as is the case in asthma, allergies, allergic rhinitis, pulmonary bronchoconstriction and hypertension, chronic obstructive pulmonary disease (COPD), pulmonary transplantation rejection, pulmonary infections, allergy, asthma, cystic fibrosis (CF), Acute Respiratory Distress Syndrome (ARDS) as well as infantile and pregnancy-related RDS, cancer, etc., which either directly or by metastasis afflict the lung, the present anti-sense oligonucleotides may be directed to a list of target mRNAs, which includes the targets listed in Table 1 above, among others.

The oligos of this invention may be obtained by first selecting fragments of a target nucleic acid having at least 4 contiguous nucleic acids selected from the group consisting of G and C and/or having a specific type and/or extent of activity, and then obtaining a first oligonucleotide 4 to 60 nucleotides long which comprises the selected fragment and has a thymidine (T) nucleic acid content

of up to and including about 15%, preferably, about 12%, about 10%, about 7%, about 5%, about 3%, about 1%, and more preferably no thymidine. The latter step may be conducted by obtaining a second oligonucleotide 4 to 60 nucleotides long comprising a sequence which is anti-sense to the selected fragment, the second oligonucleotide having an adenosine base content of up to and including about 15%, preferably about 12%, about 10%, about 7%, about 5%, about 3%, about 1%, and more preferably no adenosine. When the selected fragment comprises at least one thymidine base, an adenosine base may be substituted in the corresponding anti-sense nucleotide fragment with a universal base selected from the group consisting of heteroaromatic bases which bind to a thymidine base but have less than about bout 10%, preferably less than about 1%, and more preferably less than about 0.3% of the adenosine base agonist activity at the adenosine A_1, A_{2a}, A_{2b} and A_3 receptors, and heteroaromatic bases which have no activity at the adenosine A_{2a} receptor, when validating in the respiratory system. Other adenosine activities in other systems may be determined in other systems, as appropriate. The analogue heteroaromatic bases may be selected from all pyrimidines and purines, which may be substituted by O, halo, NH_2, SH, SO, SO_2, SO_3, COOH and branched and fused primary and secondary amino, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, alkoxy, alkenoxy, acyl, cycloacyl, arylacyl, alkynoxy, cycloalkoxy, aroyl, arylthio, arylsulfoxyl, halocycloalkyl, alkylcycloalkyl, alkenylcycloalkyl, alkynylcycloalkyl, haloaryl, alkylaryl, alkenylaryl, alkynylaryl, arylalkyl, arylalkenyl, arylalkynyl, arylcycloalkyl, which may be further substituted by O, halo, NH_2, primary, secondary and tertiary amine, SH, SO, SO_2, SO_3, cycloalkyl, heterocycloalkyl and heteroaryl. The pyrimidines and purines may be substituted at all positions as is known in the art, but preferred are those which are substituted at positions 1, 2, 3, 4, 7 and/or 8. More preferred are pyrimidines and purines such as theophylline, caffeine, dyphylline, etophylline, acephylline piperazine, bamifylline, enprofylline and xantine having the chemical formula



wherein R^1 and R^2 are independently H, alkyl, alkenyl or alkynyl and R^3 is H, aryl, dicycloalkyl, dicycloalkenyl, dicycloalkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, O-cycloalkyl, O-cycloalkenyl, O-cycloalkynyl, NH_2-alkylamino-ketoxyalkyloxy-aryl, mono and dialkylaminoalkyl-N-alkylamino-SO_2aryl, among others. Similar modifications in the sugar are also embodiments of this invention. Reduced adenosine content of the anti-sense oligos corresponding to the thymidines (T) present in the target RNA serves to prevent the breakdown of the oligos into products that free adenosine into the system, e.g. the lung, brain, heart, kidney, etc., tissue environment and, thereby, to prevent any unwanted effects due to it. By means of example, the Nf6B transcription factor may be selected as a target, and its mRNA or DNA searched for low thymidine (T) or desthymidine (desT) fragments. Only desT segments of the mRNA or DNA are selected which, in turn, will produce desA anti-sense as their complementary strand. When a number of RNA desT segments are found, the sequence of the anti-sense segments may be deduced. Typically, about 10 to 30 and even larger numbers of desA anti-sense sequences may be obtained. These anti-sense sequences may include some or all desA anti-sense oligonucleotide sequences corresponding to desT segments of the mRNA of the target, such as anyone of those shown in Table 1 above, in Table 2 below, and others associated with functions of the brain, cardiovascular and renal systems, and many others. When this occurs, the anti-sense oligonucleotides found are said to be 100% A-free. For each of the original desA anti-sense oligonucleotide sequences corresponding to the target gene, e.g. the NF6B transcription factor, typically about 10 to 30 sequences may be found within the target gene or RNA which have a low content of thymidine (RNA). In accordance with this invention, the selected fragment sequences may also contain a small number of thymidine (RNA) nucleotides within the secondary or tertiary or quaternary sequences. In some cases,

a large adenosine content may suffice to render the anti-sense oligonucleotide less active or even inactive against the target. In accordance with this invention, these so called "non-fully desA" sequences may preferably have a content of adenosine of less than about 15%, about 12%, about 10%, about 7%, about 5%, and about 2% adenosine. Most preferred is no adenosine content (0%). In some instances, however, a higher content of adenosine is acceptable and the oligonucleotides still fail to show detrimental "adenosine activity". A particular important embodiment is that where the adenosine nucleotide is "fixed" or replaced by a "Universal or alternative" base that may base-pair with similar or equal affinity to two or more of the four nucleotide present in natural DNA: A, G, C, and T.

A universal or alternative base is defined in this patent as any compound, more commonly an adenosine analogue, which has substantial capacity to hybridize to thymidine, while at the same time having reduced, or substantially lacking, ability to bind adenosine receptors or other molecules through which adenosine may exert an undesirable side effect in the experimental animal or in a cell system. Alternatively, adenosine analogs which completely fail to activate, or have significantly reduce ability for activating, adenosine receptors, such as the adenosine A_1, A_{2b} and/or A_3 receptors, most preferably A_1 receptors, and those that may even act as agonists of the adenosine A_{2a}, receptor, may be used. One example of a universal base is α-deoxyribofuranosol-(5-nitroindole), and an artisan will know how to select others. This "fixing" step generates further novel sequences, different from those anti-sense to the ones found in nature, that permits the anti-sense oligonucleotide to bind, preferably equally well, with the target RNA. Other examples of universal or alternative bases are 2-deoxyribosyl-(5-nitroindole). Other examples of universal bases are 3 - nitropyrrole - 2' - deoxynucleoside, 5 - nitro-indole, 2 - deoxyribosyl - (5 - nitroindole), 2-deoxyribofuranosyl - (5-nitroindole), 2' - deoxyinosine, 2' -deoxynebularine, 6H, 8H-3,4-dihydropyrimido [4, 5 - c] oxazine - 7 - one and 2 - amino - 6 -methoxy aminopurine. In addition to the above, Universal bases which may be substituted for any other base although with somewhat reduced hybridization potential, include 3 - nitropyrrole 2' - deoxynucleoside 2 - deoxyribofuranosyl - (5 - nitroindole), 2' - deoxyinosine and 2' - deoxynebularine (Glen Research, Sterling, VA). More specific mismatch repairs may be made using "P" nucleotide, 6H, 8H - 3, 4 - dihydropyrimido [4,5 - c] [1, 2] oxazin - 7 - one, which base pairs with either guanine (G) or adenine (A) and "K" nucleotide, 2 - amino - 6 - methoxyaminopurine, which base pairs with either cytidine (C) or thymidine (T), among others. Others which are known in the art or will become available are also suitable. See, for example, Loakes, D. and Brown, D. M., Nucl. Acids Res. 22:4039-4043 (1994); Ohtsuka, E. et al., J. Biol. Chem.260(5):2605-2608 (1985); Lin, P.K.T. and Brown, D. M., Nucleic Acids Res. 20(19):5149-5152 (1992; Nichols, R. et al., Nature 369(6480): 492-493 (1994); Rahmon , M. S. and Humayun, N. Z., Mutation Research 377 (2): 263-8 (1997); Amosova, O., et al., Nucleic Acids Res. 25 (!0): 1930-1934 (1997); Loakes D. & Brown, D. M., Nucleic Acids Res. 22 (20): 4039-4043 (1994), the entire sections relating to universal bases and their preparation and use in nucleic acid binding being incorporated herein by reference. When non-fully desT sequences are found in the naturally occurring target, they typically are selected so that about 1 to 3 universal base substitutions will suffice to obtain a 100% "desA" anti-sense oligonucleotide. Thus, the present method provides either anti-sense oligonucleotides to different targets which are low in, or devoid of, A content, as well as anti-sense oligonucleotides where one or more adenosine nucleotides, e. g. about 1 to 3, or more, may be "fixed" by replacement with a "Universal" or "replacement" base. Universal bases are known in the art and need not be listed herein. An artisan will know which bases may act as universal bases, and replace them for A.. Table 2 below provides a selected number of targets to which the agents of the invention are effectively applied. Others, however, may also be targeted.

Table 2: Cancer Targets

Transforming Oncogenes	Therapy Targets
ras	thymidylate synthetase
src	thymidylate synthetase
myc	dihydrofolate reductase

bcl-2	thymidine kinase
	deoxycytidine kinase
	ribonucleotide reductase
Angiogenesis factors	Adhesion Molecules
Oncogenes	Folate Pathway Enzymes
DNA repair genes	(One Carbon Pool)
	Telomerase
	HMG CoA Reductase
	Farnesyl Transferase
	Glucose-6-Phosphate Transferase

A group of preferred targets for the treatment of cancer are genes associated with any of different types of cancers, or those generally known to be associated with malignancies, whether they are regulatory or involved in the production of RNA and/or proteins. Examples are transforming oncogenes, including, but not limited to, ras, src, myc, and BCL-2, among others. Other targets are those to which present cancer chemotherapeutic agents are directed to, such as various enzymes, primarily, although not exclusively, thymidylate synthetase, dihydrofolate reductase, thymidine kinase, deoxycytidine kinase, ribonucleotide reductase, and the like. The present technology is particularly useful in the treatment of cancer ailments given that traditional cancer therapies are fraught with the unresolved problem of selectively killing cancer cells while preserving normal living cells from the devastating effects of treatments such as chemotherapy, radiotherapy, and the like. The present technology provides the ability of selectively attenuating or enhancing a desired pathway or target. This approach provides a significant advantage over standard treatments of cancer because it permits the selection of a pathway, including primary, secondary and possibly tertiary targets, which are not generally expressed simultaneously in normal cells. Thus, the present agent may be administered to a subject to cause a selective increase in toxicity within tumor cells that, for instance, express all three targets while normal cells that may expresses only one or two of the targets will be significantly less affected or even spared. A group of preferred targets for the treatment of cancers are genes associated with different types of cancers, or those generally known to be associated with malignancies, whether they are regulatory or involved in the production of RNA and/or proteins. Examples are transforming oncogenes, including, but not limited to, ras, src, myc, and BCL-2, among others. Other targets are those to which present cancer chemotherapeutic agents are directed to, such as various enzymes, primarily, although not exclusively, thymidylate synthetase, dihydrofolate reductase, thymidine kinase, deoxycytidine kinase, ribonucleotide reductase, and the like.

In one embodiment, at least one of the mRNAs to which the oligo of the invention is targeted encodes a protein such as transcription factors, stimulating and activating factors, intracellular and extracellular receptors and peptide transmitters in general, interleukins, interleukin receptors, chemokines, chemokine receptors, endogenously produced specific and non-specific enzymes, immunoglobulins, antibody receptors, central nervous system (CNS) and peripheral nervous and non-nervous system receptors, CNS and peripheral nervous and non-nervous system peptide transmitters, adhesion molecules, defensines, growth factors, vasoactive peptides and receptors, and binding proteins, among others; or the mRNA is corresponding to an oncogene and other genes associated with various diseases or conditions. Examples of target proteins are eotaxin, major basic protein, preproendothelin, eosinophil cationic protein, P-selectin, STAT 4, MIP-1α, MCP-2, MCP-3, MCP-4, STAT 6, c-mas, NF-IL-6, cyclophillins, PDG2, cyclosporin A-binding protein, FK5-binding protein, fibronectin, LFA-1 (CD11a/CD18), PECAM-1, C3bi, PSGL-1,CD-34, substance P, p150,95, Mac-1 (CD11b/CD18), VLA-4, CD-18/CD11a, CD11b/CD18, C5a, CCR1, CCR2, CCR4, CCR5, and LTB-4, among others. Others are, however, suitable, as well. In another embodiment, at least one of the mRNAs to which the oligo is targeted encodes intracellular and extracellular receptors and peptide transmitters such as sympathomimetic receptors, parasympathetic receptors, GABA receptors, adenosine receptors, bradykinin receptors, insulin receptors, glucagon receptors, prostaglandin receptors, thyroid receptors, androgen receptors, anabolic receptors, estrogen receptors, progesterone

receptors, receptors associated with the coagulation cascade, adenohypophyseal receptors, adenohypophyseal peptide transmitters, and histamine receptors (HisR), among others. However others are also contemplated. The encoded sympathomimetic receptors and parasympathomimetic receptors include acetylcholinesterase receptors (AcChaseR) acetylcholine receptors (AcChR), atropine receptors, muscarinic receptors, epinephrine receptors (EpiR), dopamine receptors (DOPAR), and norepinephrine receptors (NEpiR), among others. Further examples of encoded receptors are adenosine A_1 receptor, adenosine A_2B receptor, adenosine A_3 receptor, endothelin receptor A, endothelin receptor B, IgE high affinity receptor, muscarinic acetylcholine receptors, substance P receptor, histamine receptor, CCR-1 CC chemokine receptor, CCR-2 CC chemokine receptor, CCR-3 CC chemokine receptor (Eotaxin Receptor), interleukin-1β receptor (IL-1βR), interleukin-1 receptor (IL-1R), interleukin-1β receptor (IL-1βR), interleukin-3 receptor (IL-3R), CCR-4 CC chemokine receptor, cysteinyl leukotriene receptors, prostanoid receptors, GATA-3 transcription factor receptor, interleukin-1 receptor (IL-1R), interleukin-4 receptor (IL-4R), interleukin-5 receptor (IL-5R), interleukin-8 receptor (IL-8R), interleukin-9 receptor (IL-9R), interleukin-11 receptor (IL-11R), bradykinin B2 receptor, sympathomimetic receptors, parasympathomimetic receptors, GABA receptors, adenosine receptors, bradykinin receptors, insulin receptors, glucagon receptors, prostaglandin receptors, thyroid receptors, androgen receptors, anabolic receptors, estrogen receptors, progesterone receptors, receptors associated with the coagulation cascade, adenohypophyseal receptors, and histamine receptors (HisR). Others are also contemplated even though not listed herein. The encoded enzymes for development of the oligos of the invention include synthetases, kinases, oxidases, phosphatases, reductases, polysaccharide, triglyceride, and protein hydrolases, esterases, elastases, and , polysaccharide, triglyceride, lipid, and protein synthases, among others. Examples of target enzymes are tryptase, inducible nitric oxide synthase, cyclooxygenase (Cox), MAP kinase, eosinophil peroxidase, β2-adrenergic receptor kinase, leukotriene c-4 synthase, 5-lipooxygenase, phosphodiesterase IV, metalloproteinase, tryptase, CSBP/p38 MAP kinase, neutrophil elastase, phospholipase A_2, cyclooxygenase 2 (Cox-2), fucosyl transferase, chymase, protein kinase C, thymidylate synthetase, dihydrofolate reductase, thymidine kinase, deoxycytidine kinase, and ribonucleotide reductase, among others. Any enzyme associated with a disease or condition, however, is suitable as a target for this invention. Suitable encoded factors for application of this invention are, among others, Nf6B transcription factor, granulocyte macrophage colony stimulating factor (GM-CSF), AP-1 transcription factor, GATA-3 transcription factor, monocyte activating factor, neutrophil chemotactic factor, granulocyte/macrophage colony-stimulating-factor (G-CSF), NFAT transcription factors, platelet activating factor, tumor necrosis factor α (TNF α), and basic fibroblast growth factor (BFGF). Additional factors are also within the invention even though not specifically mentioned. Suitable adhesion molecules for use with this invention include intracellular adhesion molecules 1 (ICAM-1), 2 (ICAM-2) and 3 (ICAM-3), vascular cellular adhesion molecule (VCAM), endothelial leukocyte adhesion molecule-1 (ELAM-1), neutrophil adherence receptor, mad CAM-1, and the like. Other known and unknown factors (at this time) may also be targeted herein. Among the cytokines, lymphokines and chemokines preferred are interleukin-1 (IL-1), interleukin-1β (IL-1β), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-11 (IL-11),CCR-5 CC chemokine, and Rantes. Others, however, may also be targeted, as they are known to be involved in specific diseases or conditions to be treated, or for their generic activities, such as inflammation. Examples of defensins for the practice of this invention are defensin 1, defensin 2, and defensin 3, and of selectins are α4β1 selectin, α4β7 selectin, LFA-1 selectin, E-selectin, P-selectin, and L-selectin. Examples of oncogenes, although not an all inclusive list, are ras, src, myc, and bcBCL. Others, however, are also suitable for use with this invention.

The agents administered in accordance with this invention are preferably designed to be anti-sense to target genes and/or mRNAs related in origin to the species to which it is to be administered. When treating humans, the agents are preferably designed to be anti-sense to a human gene or RNA. The agents of the invention encompass oligonucleotides which are anti-sense to naturally occurring

DNA and/or RNA sequences, fragments thereof of up to a length of one (1) base less than the targeted sequence, preferably at least about 7 nucleotides long, oligos having only over about 0.02%, more preferably over about 0.1%, still more preferably over about 1%, and even more preferably over about 4% adenosine nucleotides, and up to about 30%, more preferably up to about 15%, still more preferably up to about 10% and even more preferably up to about 5%, adenosine nucleotide, or lacking adenosine altogether, and oligos in which one or more of the adenosine nucleotides have been replaced with so-called universal bases, which may pair up with thymidine nucleotides but fail to substantially trigger adenosine receptor activity. Examples of human sequences and fragments, which are not limiting, of anti-sense oligonucleotide of the invention are the following fragments as well as shorter segments of the fragments and of the full gene or mRNA coding sequences, exons and intron-exon junctions encompassing preferably 7, 10, 15, 18 to 21, 24, 27, 30, n-1 nucleotides for each sequence, where n is the sequence=s total number of nucleotides. These fragments may be selected from any portion of the longer oligo, for example, from the middle, 5'- end, 3'- end or starting at any other site of the original sequence. Of particular importance are fragments of low adenosine nucleotide content, that is, those fragments containing less than or about 30%, preferably less than or about 15%, more preferably less than or about 10%, and even more preferably less than or about 5%, and most preferably those devoid of adenosine nucleotide, either by choice or by replacement with a universal base in accordance with this invention. The agent of the invention includes as a most preferred group sequences and their fragments where one or more adenosines present in the sequence have been replaced by a universal base (B), as exemplified here. Similarly, also encompassed are all shorter fragments of the B-containing fragments designed by substitution of B(s) for adenosine(s) (A(s)) contained in the sequences, fragments thereof or segments thereof, as described above. A limited list of sequences and fragments is provided below.

Some of the examples of anti-sense oligonucleotide sequence fragments target the initiation codon of the respective gene, and in some cases adenosine is substituted with a universal or alternative base adenosine analogue denoted as "B", which lacks ability to bind to the adenosine A_1 and/or A_3 receptors. In fact, such replacement nucleotide acts as a "spacer". Many of the examples shown below provide one such sequence and many fragments overlapping the initiation codon, preferably wherein the number of nucleotides n is about 7, about 10, about 12, about 15, about 18, about 21 and up to about 28, about 35, about 40, about 50, about 60.

Human Receptor-related Antisense Polynucleotide

5'-GGCGGCCTGG AAAGCTGAGA TGGAGGGCGG CATGGCGGGC ACAGGCTGGG C TGCTTTTCT TTTCTGGGCC TCTGTGGTCT GTTTTTTTCT GGCCCTGCTG GGGCGCTCTC CGCCGCCCGC CTGGCTCCCG GBGCCCBTGB TGGGCBTGCC GTGGTTCTTG CCCTCCTTTG GCTGCCGTGC CCGCTCCCCG GCCTCCTGGC GGGTGGCCGT TGGGCCCGTG TTCCCCTGGG GCCTGGGGCT CCCTTCTCTC GCCCTTCTTG CTGGGCCTCT GCTGCTGCTG GTGCTGTGGC CCCCGTACA CCGAGGAGCC CATGATGGGC ATGCCACAGA CGACAGGCGT BCBCCGBGGB GCCCBTGBTG GGCBTGCCBC BGBCGBCBGG C GGC GCC GTG CCG CGT CTT GGT GGC GGC GG GTT CGC GCC CGC GCG GGG CCC CTC CGG TCC GTT CGC GCC CGC GCG GGG CCC CTC CGG TCC CGG GTC GGG GCC CCC CGC GGC C GCC TCG GGG CTG GGG CGC TGG TGG CCG GG CCG CGC CTC CGC CTG CCG CTT CTG GCT GGG CCC CGG GCG CCC CCT CCC CTC TTG CTC GGG TCC CCG TG ACA GCG CGT CCT GTG TCT CCA GCA GCA TGG CCG GGC CAG CTG GGC CCC BCB GCG CGT CCT GTG TCT CCB GCB GCB TGG CCG GGC CBG CTG GGC CCC ACA GAG CAG TGC TGT TGT TGG GCA TCT TGC CTT CCC AGG G BCB GBG CB TGC TGT TGT TGG GCB TCT TGC CTT CCC BGG GCC CTT TTC TGG TGG GGT GGT GCT GTT GTT GGG CTT TCT TCT GTT CCC BCB GBG CBG TGC TGT TGT TGG GCB TCT TGC CTT CCC BGG GCC CTT TTC TGG TGG GGT GGT GCT GTT GTT GGG C TTT CTT CTG TTC CC TTT CCC CTG GGT CTT CC CTC CTG CTC TTT TTT C ATT TGC TCT CCT ATT ACT TTC TGT GTC CAT TTT TTC ATT AAC CGA GCT GT BTT TGC TCT CCT BTT BCT TTC TGT GTC CBT TTT TTC BTT BBC CGB GCT GT GCC TGT GTC TGT CCT CCT GCT TCG TTC CTC TCG TTC CTG CTT GGT GCC CTT GCC G GTC CTG CTC CTC CGG GCT GTG G GTC GTG GCC CTG GCT CCG GCT GGT GGG CTC CCC TGG CCT TCG CTG GCT GGC GGC GTG C GGG TCT TGC TCT GGG CCT GGC TGT GGC CGT GGT TGG GGG TCT TC GCT GCC TCC GTT TGG GTG GC TCT CTG AAT ATT GAC CTT CCT CCA TGG CGG TCC TGC TTG GAT TCT CCC GA TCT CTG BBT BTT GBC CTT CCT CCB TGG CGG TCC TGC TTG GBT TCT CCC GB GCC TTT CCT GGT TCT CTT GTT GTT TTT GGG GTT TGG CTT ACA GTA GAG TAG GGG ATT CCA TGG CAG GAG CCA TCT

AGTCCAGTAA CACAGACAGT GCAGGGGCCC TGGGCACCCT CAGGTTCTGT GTGTTCGGGC TCGGCTCCCG
GGCATACCCC CACTTCTGCG CCTTTGCTCG TGCCGTGGAC ACACGGCTGG AGGAACTGGG CGGGGAGCGG
CTGCTGCAGC TGGGCCAGGG CGACGAGCTG TGCGGCCAGG AGGAGGCCTT CCGAGGCTGG GCCCAGGCTG
CCTTCCAGGC CGCCTGTGAG ACCTTCTGTG TGGGAGAGGA TGCCAAGGCC GCCGCCCGAG ACATCTTCAG
CCCCAAACGG AGCTGGAAGC GCCAGAGGTA CCGGCTGAGC GCCCAGGCCG AGGGCCTGCA GTTGCTGCCA
GGTCTGATCC ACGTGCACAG GCGGAAGATG TTCCAGGCTA CAATCCGCTC AGTGGAAAAC CTGCAAAGCA
GCAAGTCCAC GAGGGCCACC ATCCTGGTGC GCCTGGACAC CGGAGGCCAG GAGGGGCTGC AGTACCAGCC
GGGGGACCAC ATAGGTGTCT GCCCGCCCAA CCGGCCCGGC CTTGTGGAGG CGCTGCTGAG CCGCGTGGAG
GACCCGCCGG CGCCCACTGA GCCCGTGGCA GTAGAGCAGC TGGAGAAGGG CAGCCCTGGT GGCCCTCCCC
CCGGCTGGGT GCGGGACCCC CGGCTGCCCC CGTGCACGCT GCGCCAGGCT CTCACCTTCT TCCTGGACAT
CACCTCCCCA CCCAGCCCTC AGCTCTTGCG GCTGCTCAGC ACCTTGGCAG AAGAGCCCAG GGAACAGCAG
GAGCTGGAGG CCCTCAGCCA GGATCCCCGA CGCTACGAGG AGTGGAAGTG GTTCCGCTGC CCCACGCTGC
TGGAGGTGCT GGAGCAGTTC CCGTCGGTGG CGCTGCCTGC CCCACTGCTC CTCACCCAGC TGCCTCTGCT
CCAGCCCCGG TACTACTCAG TCAGCTCGGC ACCCAGCACC CACCCAGGAG AGATCCACCT CACTGTAGCT
GTGCTGGCAT ACAGGACTCA GGATGGGCTG GGCCCCCTGC ACTATGGAGT CTGCTCCACG TGGCTAAGCC
AGCTCAAGCC CGGAGACCCT GTGCCCTGCT TCATCCGGGG GGCTCCCTCC TTCCGGCTGC CACCCGATCC
CAGCTTGCCC TGCATCCTGG TGGGTCCAGG CACTGGCATT GCCCCCTTCC GGGGATTCTG GCAGGAGCGG
CTGCATGACA TTGAGAGCAA AGGGCTGCAG CCCACTCCCA TGACTTTGGT GTTCGGCTGC CGATGCTCCC
AACTTGACCA TCTCTACCGC GACGAGGTGC AGAACGCCCA GCAGCGCGGG GTGTTTGGCC GAGTCCTCAC
CGCCTTCTCC CGGGAACCTG ACAACCCCAA GACCTACGTG CAGGACATCC TGAGGACGGA GCTGGCTGCG
GAGGTGCACC GCGTGCTGTG CCTCGAGCGG GGCCACATGT TTGTCTGCGG CGATGTTACC ATGGCAACCA
ACGTCCTGCA GACCGTGCAG CGCATCCTGG CGACGGAGGG CGACATGGAG CTGGACGAGG CCGGCGACGT
CATCGGCGTG CTGCGGGATC AGCAACGCTA CCACGAAGAC ATTTTCGGGC TCACGCTGCG CACCCAGGAG
GTGACAAGCC GCATACGCAC CCAGAGCTTT TCCTTGCAGG AGCGTCAGTT GCGGGGCGCA GTGCCCTGGG
CGTTCGACCC TCCCGGCTCA GACACCAACA GCCCCTGAGA GCCGCCTGGC TTTCCCTTCC AGTTCCGGGA
GAGCGGCTGC CCGACTCAGG TCCGCCCGAC CAGGATCAGC CCCGCTCCTC CCCTCTTGAG GTGGTGCCTT
CTCACATCTG TCCAGAGGCT GCAAGGATTC AGCATTATTC CTCCAGGAAG GAGCAAAACG CCTCTTTTCC
CTCTCTAGGC CTGTTGCCTC GGGCCTGGGT CCGCCTTAAT CTGGAAGGCC CCTCCCAGCA GCGGTACCCC
AGGGCCTACT GCCACCCGCT TCCTGTTTCT TAGTCCGAAT GTTAGATTCC TCTTGCCTCT CTCAGGAGTA
TCTTACCTGT AAAGTCTAAT CTCTAAATCA AGTATTTATT ATTGAAGATT TACCATAAGG GACTGTGCCA
GATGTTAGGA GAACTACTAA AGTGCCTACC CCAGCTC-3' **(SEQ. ID NO:3003)**

Human Factor Related Anti-sense Oligonucleotide

5'-CCT CCT TCC TGG TCT GTC TGC CBG BCB BBT TTG GGB BGT GBB CBG TTT TGG BBC CBT GTT TCC CBG
TCT CTG BGC TGT GGC GCC CTG CTG CTC TTT CTG CT TCC CTT GGT GGG TTG GGC C GCT GGT TGT TCT
GGG GTT C TTG CTG CCC CTT CTG TCC C TGT TTG CTG GTG TCT GCG C CCC CBB CBG BBG BBG CBG BCB
BBT TTG GGB BGT GBB CBG TTT TGG BBC CBT GTT TCC TGT GCG CTC GGC CTG GTC CCG G GGG TCT CCT
CTT GTT GTT GC TTG CGC CTC CTG CTG GGG GT CC CTC TGT TCT TGT TTT GGG GGC GGG CCC GGC CGT
TGT CTT G GTT TGG GGG TTT CCG TTG GGG TTC TCC TGG CCC GGG CCT TGC CC GGC CGT GGT CCC
GGC TTC GTTCCT GTC TCC GTC TCG GCT CTT CTG GGG CCT TGC GCT GTC TTT GGT G GCB CCG TCC
BGT GBT GGT GCG GTB CTT GTC GCT GCB GCG CTC GGC CTG GTC CCG GBG BGC GCG CGG GCC GGG GGC
TGC TGG G GGT TGG CCC GGG GTG CCC C GCC GCT GGG TGC CCT CGT CCT CTG CGG TC GTG TCT CCT
GGC TCT GGT TCC CC GCT GCG CCC GTT GTC CTC TGG GGT GGC CTT C GCT CCC GGG TCT GGT TCT TGT
GT TGG GGG TCC CTT TTT GGG CCT GTT GT GGC GTG GCT TGT GTG TTC GGT TTC TGC CCT GTC CTC CGG
CGT CCC CGG BGC CTC CCC GGG GCB GGB TGB CTT TTG BGG GGG BCB CBG BTG TCT GGG CBT TGC CBG
GTC CTG GGB BCB GBG CCC CGB GCB GGB CCB GGB GTG CGG GCB GCG CGG GCC GGG GGC TGC TGG GBG
CCB TBG CGB GGC TGB G CCT CTT TTC TGT TTT TCC C CTC TGC CTT TGT TTG GGT TCG CTT CCT TTC
TGC TTC TTC C CTG TGT CTC CTG TCT CCG CTT TTT TCT TC GTC TTT GTT GTT TTC TCT TCC TTG CTG
BGC BBG BTB TCT BGB TTC TGG GGT GGT CTC GBT TTT BBBB GCT TGB GBB GCT GCB BBC BTT BTC CBB
BGT BTB TTT GBG GCT CCB BGG BTC BCG BCC BTC TTC CCB GGC BTT TTB BGT TGC TGT CGT BBG TGB
GBG CTG BGB GBB BCT GTG BBG CBB TCB TGB CTT CBB GBG TTC TTT TCB CCC GTT CTT GGC TTC TTC
TGT C CGT TGG CTT CTC GTT GTC CC TGT GGG CTT CTC GTT GTC CC CCC TTC GGG GGC TGG TGG GGC
CGT CCT TGC CTG CTG G GTT CTT GGC TTC TTC TGT CCG T TGG CTT CTC GTT GTC CC TGT GGG CTT
CTC GTT GTC CC CCC TTC GGG GGC TGG TGG GGC CGT CCT TGC CTG CTG G TTT TCT CTT TCG CTT TCT
TTT CGT CTC CTG TTC CTC CTT TT TTG CTG TTT TTT CTC CTT CTT CTC TCC TTT CTT TTC TTT TCT CTT
TCG CTT TCT TTT CGT CTC CTG TTC CTC CTT TT TTG CTG TTT TTT CTC CTT CTT CTC TCC TTT CTT TTC
CTC TGT CTT GTT CTG GTC CTT CGT GGG GCT CTG TGT CGC GTG G GTG CGG CCG TGG CC GGC GGB CCB
GGB GTT GGB GCB GGB GCB GGB CGG GCB GGC GGC TCB TGT TTG GBT CGG CBG GBG GCB CTC CTC TGG
TTG GCT TCC TTC GCC GGC BCB TGC TBG CBG GBB GBB CBG BGG GGG BBG CBG TTG GGB GGT GBG BCC
CBT TBB TBG GTG TCG B TCCCTGTTTC CCCCCTTTCG TTCTGCGTTT GCCTTTGGCG TTTTTTGTTT
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GACTCCAGCC CTCACCCATG AGTTCCTGAA CTTGAACCCA GAGACCCCAT CTCTAAGACT TCAGCCTTGA
GATCCAGGGC CTGACCCTAG ACTCGAGCCC ACAGACCTCA GATACTGTCT GTAAAACCCC AGCTCTGGTG
GGGAGCAGTG GCTCACTCCT GTAATCCCAA GGCAGGGGAG GCCAAGGCAG AAGGACCTCT TGAGGCCATG
AGTTTGAGAC AGCCTGGGCA GCATAGCAAG ACTCTGTTTC TTAATTATTA TTATTATTAT TATTTTTGG
AGACAGAGTC TCGCGCTCTG TTGCCCAGGC TAGAGTGCAA TGGTGCCATT TCGGCTTGCT GGAACCTCCG
CCTCCTGGGC TCAAGCGATT CTCCTGCCTC AGCCTCCTGA GTAGCTGGGA CTTCAGGTGC ACACTGCCAC
ACCCGGATAA TTTTTTTGTA TTTTAGTAGA CACAGGGTTT CACCGTGTTG CCCAGGCTGG TCACAAACTC
CTGAGCTCAG GCCATCCGCC CGCCTCGGCC TCCCAAAGCG CTGGGATAAC AGGCGTGACG CCGCGCCTGG
CTTCTTAATT GTTCTAACAG CAGCGACAAC AACAAAAACC CAGCTCTGAG ATTCCAGCCC CGGCGACTCT
AACAGTCCCA GGCCCGATCC CTCACCTAGA ACCGAGATGC CAGCCCTGAC TCCACAGACT TCACCCCCAA
CCCCCACACT CAGCTCTGGA AGCCCGTCCT GACTCCAGCC TCCATTTTCG GAACCCCACA GCCTGAAGAG
CTCCCGGCCT AAACACTTCA CCCCACGCGC CACAGTCCCC CTGTGAATAT GCAGCCCCGA TTCAGCTGCA
GCTCCACAGC ACCCCTGCCC TGCACCCCCG CTGCACCCCC TACCTGTGAC TCACCTCTCT CCTCTCCCCA
CAGATGTCCC GCCTGGCCCT GCCCCAGCCA CCCCCGGACC CGCCGGCGCC CCGCGCTGGCG CCCCCCTCCT
CAGCCTGGGG GGGCATCAGG GCCGCCCACG CCATCCTGGG GGGGCTGCAC CTGACACTTG ACTGGGCCGT
GAGGGGACTG CTGCTGCTGA AGACTCGGCT GTGACCCGGG GCCCAAAGCC ACCACCGTCC TTCCAAAGCC
AGATCTTATT TATTTATTTA TTTCAGTACT GGGGGCGAAA CAGCCAGGTG ATCCCCCCGC CATTATCTCC
CCCTAGTTAG AGACAGTCCT TCCGTGAGGC CTGGGGGGCA TCTGTGCCTT ATTTATACTT ATTTATTTCA
GGAGCAGGGG TGGGAGGCAG GTGGACTCCT GGGTCCCCGA GGAGGAGGGG ACTGGGGTCC CGGATTCTTG
GGTCTCCAAG AAGTCTGTCC ACAGACTTCT GCCCTGGCTC TTCCCCATCT AGGCCTGGGC AGGAACATAT
ATTATTTATT TAAGCAATTA CTTTTCATGT TGGGGTGGGG ACGGAGGGGA AAGGGAAGCC TGGGTTTTTG
TACAAAAATG TGAGAAACCT TTGTGAGACA GAGAACAGGG AATTAAATGT GTCATACATA TCCACTTGAG
GGCGATTTGT CTGAGAGCTG GGGCTGGATG CTTGGGTAAC TGGGGCAGGG CAGGTGGAGG GGAGACCTCC
ATTCAGGTGG AGGTCCCGAG TGGGCGGGGC AGCGACTGGG AGATGGGTCG GTCACCCAGA CAGCTCTGTG
GAGGCAGGGT CTGAGCCTTG CCTGGGGCCC CGCACTGCAT AGGGCCGTTT GTTTGTTTTT TGAGATGGAG
TCTCGCTCTG TTGCCTAGGC TGGAGTGCAG TGAGGCAATC TAAGGTCACT GCAACCTCCA CCTCCCGGGT
TCAAGCAATT CTCCTGCCTC AGCCTCCCGA TTAGCTGGGA TCACAGGTGT GCACCACCAT GCCCAGCTAA
TTATTTATTT CTTTTGTATT TTTAGTAGAG ACAGGGTTTC ACCATGTTGG CCAGGCTGGT TTCGAACTCC
TGACCTCAGG TGATCCTCCT GCCTCGGCCT CCCAAAGTGC TGGGATTACA GGTGTGAGCC ACCACACCTG
ACCCATAGGT CTTCAATAAA TATTTAATGG AAGGTTCCAC AAGTCACCCT GTGATCAACA GTACCCGTAT
GGGACAAAGC TGCAAGGTCA AGATGGTTCA TTATGGCTGT GTTCACCATA GCAAACTGGA AACAATCTAG
ATATCCAACA GTGAGGGTTA AGCAACATGG TGCATCTGTG GATAGAACGC CACCCAGCCG CCCGGAGCAG
GGACTGTCAT TCAGGGAGGC TAAGGAGAGA GGCTTGCTTG GGATATAGAA AGATATCCTG ACATTGGCCA
GGCATGGTGG CTCACGCCTG TAATCCTGGC ACTTTGGGAG GACGAAGCGA GTGGATCACT GAAGTCCAAG
AGTTTGAGAC CGGCCTGCGA GACATGGCAA AACCCTGTCT CAAAAAAGAA AGAATGATGT CCTGACATGA
AACAGCAGGC TACAAAACCA CTGCATGCTG TGATCCCAAT TTTGTGTTTT TCTTTCTATA TATGGATTAA
AACAAAAATC CTAAAGGGAA ATACGCCAAA ATGTTGACAA TGACTGTCTC CAGGTCAAAG GAGAGAGGTG
GGATTGTGGG TGACTTTTAA TGTGTATGAT TGTCTGTATT TTACAGAATT TCTGCCATGA CTGTGTATTT
TGCATGACAC ATTTTAAAAA TAATAAACAC TATTTTTAGA ATAACAGAAT ATCAGCCTCC TCCTCTCCAA
AAATAAGCCC TCAGGAGGGG ACAAAGTTGA CCGCTGATTG AGCCTGTCAG GGCTGTGCAC-3' (SEQ. ID NO:3004)

Human Adenosine A_1 Receptor Nucleic Acid and Antisense Oligonucleotide Fragments

5'-ATGCCGCCCT CCATCTCAGC TTTCCAGGCC GCCTACATCG GCATCGAGGT GCTCATCGCC CTGGTCTCTG
TGCCCGGGAA CGTGCTGGTG ATCTGGGCGG TGAAGGTGAA CCAGGCGCTG CGGGATGCCA CCTTCTGCTT
CATCGTCTCG CTGGCGGTGG CTGATGTGGC CGTGGGTGCC CTGGTCATCC CCCTCGCCAT CCTCATCAAC
ATTGGGCCAC AGACCTACTT CCACACCTGC CTCATGGTTG CCTGTCCGGT CCTCATCCTC ACCCAGAGCT
CCATCCTGGC CCTGCTGGCA ATTGCTGTGG ACCGCTACCT CCGGGTCAAG ATCCCTCTCC GGTACAAGAT
GGTGGTGACC CCCCGGAGGG CGGCGGTGGC CATAGCCGGC TGCTGGATCC TCTCCTTCGT GGTGGGACTG
CCCCTATGT TTGGCTGGAA CAATCTGAGT GCGGTGGAGC GGGCCTGGGC AGCCAACGGC AGCATGGGGG

GGATATAGGT TTCCAATTAA GTACATGGTC AAGTATTAAC AGCACAAGTG GTAGGTTAAC ATTAGAATAG
GAATTGGTGT TGGGGGGGGG GTTTGCAAGA ATATTTTATT TTAATTTTTT GGATGAAATT TTTATCTATT
ATATATTAAA CATTCTTGCT GCTGCGCTGC AAAGCCATAG CAGATTTGAG GCGCTGTTGA GGACTGAATT
ACTCTCCAAG TTGAGAGATG TCTTTGGGTT AAATTAAAAG CCCTACCTAA AACTGAGGTG GGGATGGGGA
GAGCCTTTGC CTCCACCATT CCCACCCACC CTCCCCTTAA ACCCTCTGCC TTTGAAAGTA GATCATGTTC
ACTGCAATGC TGGACACTAC AGGTATCTGT CCCTGGGCCA GCAGGGACCT CTGAAGCCTT CTTTGTGGCC
TTTTTTTTTT TTCATCCTGT GGTTTTTCTA ATGGACTTTC AGGAATTTTG TAATCTCATA ACTTTCCAAG
CTCCACCACT TCCTAAATCT TAAGAACTTT AATTGACAGT TTCAATTGAA GGTGCTGTTT GTAGACTTAA
CACCCAGTGA AAGCCCAGCC ATCATGACAA ATCCTTGAAT GTTCTCTTAA GAAAATGATG CTGGTCATCG
CAGCTTCAGC ATCTCCTGTT TTTTGATGCT TGGCTCCCTC TGCTGATCTC AGTTTCCTGG CTTTTCCTCC
CTCAGCCCCT TCTCACCCCT TTGCTGTCCT GTGTAGTGAT TTGGTGAGAA ATCGTTGCTG CACCCTTCCC
CCAGCACCAT TTATGAGTCT CAAGTTTTAT TATTGCAATA AAAGTGCTTT ATGCCCGAAT TC-3' **(FRAG.NO:_) (SEQ. ID NO:2497)**

5' GCCGCCGCCA TGGGAGTGCA GGTGGAAACC ATCTCCCCAG GAGACGGGCG CACCTTCCCC AAGCGCGGCC
AGACCTGCGT GGTGCACTAC ACCGGGATGC TTGAAGATGG AAAGAAATTT GATTCCTCCC GGGACAGAAA
CAAGCCCTTT AAGTTTATGC TAGGCAAGCA GGAGGTGATC CGAGGCTGGG AAGAAGGGGT TGCCCAGATG
AGTGTGGGTC AGAGAGCCAA ACTGACTATA TCTCCAGATT ATGCCTATGG TGCCACTGGG CACCCAGGCA
TCATCCCACC ACATGCCACT CTCGTCTTCG ATGTGGAGCT TCTAAAACTG GAATGACAGG AATGGCCTCC
TCCCTTAGCT CCCTGTTCTT GGATCTGCCR TGGAGGGATC TGGTGCCTCC AGACATGTGC ACATGARTCC
ATATGGAGCT TTTCCTGATG TTCCACTCCA CTTTGTATAG ACATCTGCCC TGACTGAATG TGTTCTGTCA
CTCAGCTTTG CTTCCGACAC CTCTGTTTCC TCTTCCCCTT TCTCCTCGTA TGTGTGTTTA CCTAAACTAT
ATGCCATAAA CCTCAAGTTA TTCA-3' **(FRAG. NO:_) (SEQ. ID NO:2498)**

wherein B is adenosine, or, more preferably, replaces adenosine and is an "equivame\\lent" or a "universal" base, and adenosine A_{2a} receptor agonist or only minimally antagonist, an adenosine A_{2b} receptor antagonist, an adenosine A_3 receptor antagonist, or an adenosine A_1 receptor antagonist. Similarly, adenosine (A) may always be replaced by an "alternative", "equivalent" and/or "universal" base having a small fraction, preferably less than 0.3 of the activity of adenosine at the adenosine receptor(s), as described above.

In one preferred embodiment, the links between neighboring mononucleotides are phosphodiester links. In another preferred, at least one mononucleotide phosphodiester residue of the anti-sense oligonucleotide(s) is substituted by a methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, 2'-O-methyl, methylene(methyimino), methyleneoxy (methylimino), phosphoramidate residues, and combinations thereof. The oligos having one or more phosphodiester residues substituted by one or more of the other residues are generally longer lasting, given that these residues are more resistant to hydrolysis than the phosphodiester residue. In some cases up to about 10%, about 30%, about 50%, about 75%, and even all phosphodiester residues may be substituted (100%). Typically, the multiple target anti-sense oligonucleotide (oligo) of the invention comprises at least about 7 mononucleotides, in some instances up to 60 and more mononucleotides, preferably about 10 to about 36, and more preferably about 12 to about 21 mononucleotides. However, other lengths are also suitable depending on the length of the target macromolecule. Examples of the MTA oligos of the invention are provided in Table 3 below, which includes ninety-four sequences (SEQ ID NOS.: 2316 through 2410).

Table 3: MTA Oligos, Location Targeted & Target

MTA Oligo	SEQ. ID No.	Location	Compound Targeted	Target
HUMNFKBP65A AS				
CCC GGC CCC GCC TCG TGC C	3019	5'=1	EPI 2192	
CGT CCB TGC CGC GGG CCC	3020	5'=28 (AUG)	EPI 2193	
GCC CCG CTG CTT GGG CTG CTC TGC CGG G	3021	5'=65	EPI 2194	
TCT GTG CTC CTC TCG CCT GGG	3022	5'=137	EPI 2195	
TGG TGG GGT GGG TCT TGG TGG	3023	5'=159	EPI 2196	
CTG TCC CTG GTC CTG TG	3024	5'=196	EPI 2197	
GGT CCC GCT TCT TC	3025	5'=362	EPI 2198	
GGG GTT GTT GTT GGT CTG G	3026	5'=401	EPI 2199	
TGT CCT CTT TCT GC	3026	5'=656	EPI 2200	
GCC TCG GGC CTC CC	3027	5'=697	EPI 2201	
GGC TGG GGT CTG CGT	3028	5'=769	EPI 2202	

GGC CGG GGG TCG GTG GGT CCG CTG	3029	5'=953	EPI 2203	
GGG CTG GGG TGC TGG CTT GGG G	3030	5'=1022	EPI 2204	
GGG GCT GGG GCC TGG GCC	3031	5'=1208	EPI 2205	
GCC TGG GTG GGC TTG GGG GC	3032	5'=1272	EPI 2206	
GCT GGG TCT GTG CTG TTG CC	3033	5'=1362	EPI 2207	
GTT GTG TGG GGG GCC	3034	5'= 1451	EPI 2208	
GCT GGG TCG GGG GGC CTC TGG GCT GTC	3035	5'=1511	EPI 2209	
GCC CCG GGG CCC CC	3036	5'=1550	EPI 2210	
TGG CTC CCC CCT CC	3037	5'=1772	EPI 2211	
GCT CCC CCC TTT CC	3038	5'=1863	EPI 2212	
CGG ACG AAG ACA GAG A	3039	5'=1979	EPI 2213	
GGC TTT GTG GGC TC	3040	5'=2011	EPI 2214	
GCC TGC TCT CCC CC	3041	5'=2312	EPI 2215	
CCC GGC CCC GCC BCG BBC C	3042	intron	EPI 2192-01A	HSU50136C4Synth
CCC GGC CCC GCC BCG	3043	intron	EPI 2192-01B	
CCC GGC CCC GCC BCG BBC C	3044	5'untr	EPI 2192-02A	HUMLIPOX5LO
CCC GGC CCC GCC BCG	3045	5'untr	EPI 2192-02B	
CCC GBC CCC GCC TCB BG	3046	trans	EPI 2192-03A	HSNFKBS Subunit
CCC GBC CCC GCC TC	3047	trans	EPI 2192-03B	
CCG GCC CCG CCT C	3048	5'untr	EPI 2192-04	TGFβR1
CCC GBB CCC GCB TBG TGC C	3049	5'trans	EPI 2192-05A	HSU58198I1 enhan
CCC GCB TBG TGC C	3050	5'untr	EPI 2192-05B	
CCC GGB CCC BCC BBG TGC C	3051	3'trans	EPI 2192-06	HSVECAD
CBG BBC CCG CCT CGT GCC	3052	intron	EPI 2192-07A	NFKB2
C CCG CCT CGT GCC	3053	intron	EPI 2192-07B	NFKB2
CCG GCB CCG CCT CBT GCC	3054	5'trans	EPI 2192-08	Carboxypep
CCG GCC CCG CCB CBT GCC	3055	3'trans	EPI 2192-09	HumADRA2Cα2AdrKid
CCC GBC CCC GBC TCG	3056	5'untrs	EPI 2192-10	HUMFK506B
CCC GGC CBC GBC TCG	3057	5'untrs	EPI 2192-11	HSNBARKS1βAdrKin
CCC GGC CCB GCC TBG	3058	5'UTR	EPI 2192-12	HSNFXN1(NFKB1)
CCC GGC BCB GBC TCG TBC C	3059	3'UTR	EPI 2192-13	HSILF(transcrp. Factor ILF)
CCC GGC CCC GCC BCG	3060		EPI-2192-14	NFKB/C4Syn/5-LO/ TGFBrecl MTA
CCC GGC CCC GCC BCG	3061		EPI-2192-15	NFKB/C4Syn/5-LOMTA
TCC BTG CCG CGG GC	3062	3' trans	EPI-2193-01	METOncogene
TCC BTG CCB CGG GCC	3063	3' trans	EPI-2193-02	HSFGR2(IG)
TCC BTG CCB CGG GCC	3064	mid cod	EPI-2193-03	5-LO
TCC BTG CCB CBG GCC	3065	mid cod	EPI-2193-04	HUMTK14
GTC CBT GBC GCG G	3066	3'trans	EPI-2193-05	HUMTNFR
TC CBT GBC GCG GG	3067	AUG		Probl.HUMPTCH cardiacK+channel
TCT GBG CTC CTC TBB CCT GGG	3068	intr	EPI-2195-01	humCSPAcytotox. Ser.Protease
CTG TGC BCC TBB CBC CTG GG	3069	intr	EPI-2195-02	HSINOSX08induc.NOS
TGT GBT CCB CTB GBC TGG G	3070		EPI-2195-03	HUMACHRM2musc.m2 acetylch.rec.
TCT GTB CTC BBC TCB CCT G	3071		EPI-2195-04	s86371s1 Neurokinin3Recept
TGC TCC TCB CBB CTG GG inflam.factor	3072	EPI-2195-05	HUMMIP1 Amacro	

Table 3: MTA Oligos, Location Targeted & Target (Cont'd)

MTA Oligo	SEQ. ID No.	Location	Compound Targeted	Target
CTC CTC TBG CCT GG	3073		EPI-2195-06	HSNBARKS4 β-Adr Rec Kinase
GTG CTC CBB TCB BCT GGG	3074		EPI-2195-07	HSTNFR2SO6TNF R2
GTG CBC CBB TCB CCT GGG	3075		EPI-2195-08	humfkbp fk506 binding prot.
TCT GTG CBC CTC TBG BCT	3076	exon	EPI-2195-09	HSNBARKS1β-Adr. Recept.Kinase
CTG TBB TCC TBB CBC CTG G	3077	intron	EPI-2195-10	HUMIL8
TGT GCT BBT CBC BCB TGG G	3078		EPI-2195-11	HSU50157 PDE4
GTG CBC CBC TCB CCT G	3079	intron/exon	EPI-2195-12	IL-2 R
CTG TGC BCC TCT C	3080	3'UTR	EPI-2203-05	IL-6 R HSIL6R
CBG TGC BCC BCT CBC CTG	3081	intr/ex	EPI-2203-06A	HSIL2rG6
G TGC BCC BCT CBC CTG	3082	intr/ex	EPI-2203-06B	HSIL2rG6
CBC CTC TCB CCT GGG	3083	coding	EPI-2203-07A	HUMIL71
C CTC TCB CCT GGG	3084	coding	EPI-2203-07B	IL-7 HUMIL71
GCT CCB CTC GCC T	3085	coding	EPI-2203-08	IL-6 R HSI6REC
TGC TCC TCB CGC C	3086	intron PDGF A	EPI-2303-09	Chain HUMPDGFAB
GTT GTT GBT CTG G	3087	3'utr	EPI-2199-01	GATA-4Transcrip. Factor for IL-5
GGT TGB BBT TGG TCT TGG	3088	Coding	EPI-2199-02	TNFα HUMTNFA
GGT TGT TGB TGB TCT G	3089	Far 5'UTR	EPI-2199-03	HSSUBP1G(Sub Pr)
GGG TTB BBG TTG BTC TGG	3090	Coding	EPI-2199-04	NeutrophilAdh. R HUMNARIA
GGG TTB BBG TTG BTC TGG	3091	HSHM2	EPI-2199-05	m2 Muscarinic R
TTG TTG TBG BTC TGG	3092	HUML1CAM	EPI-2199-06	L1 LeukAadhProt
GGG TBG BBG BGT CCG CTG	3093	coding	EPI-2203-01	HUMGATA2A
GGG TCB GBG GBT CBG CTG	3094	S71424S2	EPI-2203-02	IGE eps
GGG TBG GTG GGT C	3095	coding	EPI-2203-03	HSGCSFR2
GGG TCG GBG GGT CBG C	3096	HUMITGF	EPI-2203-04	TGFβ3
GGG TGG GCT T	3097	HUMNK65PRO	EPI-2206-01	NFKB/NK & TCell Activating Prot
GGG TGG GCT TGG G	3098	HUMPEREEB	EPI 2206-02	NFKB/Prostagl. EP3 Rec
CCTGGGTGGGBBTGGG	3099		EPI 2206-03	HSNF2B/GCSF NFKB/GranuLocCSF/ Transcr.FactorNF2B
CCTGGBTGGGCBTGGG	3100		EPI-2206-04	HUMLAP/NFKB Leuk.Adhes.Prot
GCCTGBGTGBBCTTGGG	3101		EPI2206-05	NFKB/Endothel N2 S63833
CCCAVGVCCVCCCAGGC	3102		EPI 2206-06	NFKBAS13/B Lymph SerThrProt.Kinase
AGCCCACCCAGGC	3103		EPI2206-07	NFKBAS13/GCSF1 HSGCSFR1Rec
BCCTGGGTGGGCTB	3104		EPI2206-08	NFKBAS13/GCSF1/ NK7TCELLACT.Prot
GGTGGGCTTGGG	3105		EPI 2206-09	NFKBAS13/ HSTGFB1 TGFB
CCBBGGTGGGCTTGGG	3106		EPI 2206-10	NFKBAS13/ HSTGFB1 TGFB1
CTGGGTGGGBBTGGG	3107		EPI 2206-11	NFKBAS13/ HSGCSFR1 GCSFR1
CCBGGGTGGGCTTGG	3108		EPI 2206-12	NFKBAS13/HUMCD30A LymphActAntigCoding
GGGTGGGCTTGG	3109		EPI-2206-12B	NFKBAS13/HUMCD30A
CCTGBGTGBGCBTGGG	3110		EPI 2206-13	NFKBAS13/HUMCAM1V Vasc.Endoth.Cell Adh.Molec

B: Universal Base

The MTA oligos of Table 3 are suitable for use with two or more of the targets listed in Table 4 below.

Table 4: Targets for the MTA Oligos of Table 3

Compound	Target
EPI 2010	Adenosine A1 receptor
EPI 2045	Adenosine A3 receptor
EPI 2873, EPI 2193	NFκB
EPI 1873	Interleukin-1
EPI 1857	Interleukin -5
EPI 2945	Interleukin -4
EPI 2977	Interleukin -8
EPI 2031	5-Lipoxygenase
EPI 1898	Leukotriene C-4 Synthase
EPI 1856	Eotaxin
EPI 1131	ICAM
EPI 1085	VCAM
EPI 2085	TNFα
EPI 1908	PAF
EPI 1925	IL-4 receptor
EPI 2643	β2 aderenergic receptor kinase
EPI 2934	Tryptase
EPI 2033	Major Basic Protein
EPI 2795	Eosinophil Peroxidase

NfκB: nuclear factor κB
ICAM: intracellular adhesion molecule
VCAM: vascular cell adhesion molecule
TNF: tumor necrosis factor
PAF: platelet activating factor

The mRNA sequence of the targeted protein may be derived from the nucleotide sequence of the gene expressing the protein, whether for existing targets or those to be found in the future. Sequences for many target genes of different systems are presently known. See, GenBank data base, NIH, the entire sequences of which are incorporated here by reference. The sequences of those genes, whose sequences are not yet available, may be obtained by isolating the target segments applying technology known in the art. Once the sequence of the gene, its RNA and/or the protein are known, anti-sense oligonucleotides are produced as described above and utilized to validate the target by in vivo administration and testing for a reduction of the production of the targeted protein in accordance with standard techniques, and of specific functions. As already described above, the anti-sense oligonucleotides may be of any suitable length, e.g., from about 7 to about 60 nucleotides in length, depending on the particular target being bound and the mode of delivery thereof. The anti-sense oligonucleotide preferably is directed to an mRNA region containing a junction between intron and exon or to regions vicinal to the junction. Where the anti-sense oligonucleotide is directed to an intron/exon junction, it may either entirely overlie the junction or may be sufficiently close to the junction to inhibit splicing out of the intervening exon during processing of precursor mRNA to mature mRNA, e.g., with the 3' or 5' terminus of the anti-sense oligonucleotide being positioned within about, for example, 10, 5, 3, or 2 nucleotide of the intron/exon junction. Also preferred are anti-sense oligonucleotides which overlap the initiation codon and, more generally, those that target the coding region of the target mRNA. When practicing the present invention, the anti-sense oligonucleotides administered may be related in origin to the species to which it is administered. When treating humans, human anti-sense may be used if desired. Anti-sense oligos to endogenous sequences from other species, however, are also encompassed.

Pharmaceutical compositions comprising an anti-sense oligonucleotide as given above effective to reduce expression of an A_1 or A_3 adenosine receptor by passing through a cell membrane and binding specifically with mRNA encoding an A_1 or A_3 adenosine receptor in the cell so as to prevent its translation are another aspect of the present invention. Such compositions are provided in a suitable pharmaceutically acceptable carrier, e.g., sterile pyrogen-free saline solution. The anti-sense oligonucleotides may be formulated with a hydrophobic carrier capable of passing through a cell membrane, e.g., in a liposome, with the liposomes carried in a pharmaceutically acceptable aqueous carrier. The oligonucleotides may also be coupled to a substance which inactivates mRNA, such as a ribozyme. Such oligonucleotides may be administered to a subject to inhibit the activation of a target, such as the adenosine receptors, which subject is in need of such treatment for any of the reasons discussed herein. Furthermore, the pharmaceutical formulation may also contain chimeric molecules comprising anti-sense oligonucleotides attached to molecules which are known to be internalized by cells. These oligonucleotide conjugates utilize cellular uptake pathways to increase cellular concentrations of oligonucleotides. Examples of macromolecules used in this manner include transferrin, asialoglycoprotein (bound to oligonucleotides via polylysine) and streptavidin. In the pharmaceutical formulation, the anti-sense compound may be contained within a lipid particle or vesicle, such as a liposome or microcrystal. The particles may be of any suitable structure, such as unilamellar or plurilamellar, so long as the anti-sense oligonucleotide is contained therein. Positively charged lipids such as N- [1-(2, 3 -dioleoyloxi) propyl] -N, N, N-trimethylammoniumethylsulfate, or "DOTAP," are particularly preferred for such particles and vesicles. The preparation of such lipid particles is well known. See, e.g., U.S. Patent Nos. 4,880,635 to Janoff et al.; 4,906,477 to Kurono et al.; 4,911,928 to Wallach; 4,917,951 to Wallach; 4,920,016 to Allen et al.; 4,921,757 to Wheatley et al.; etc.

Subjects may be administered the active composition by any means which transports the anti-sense nucleotide composition to the lung. The anti-sense compounds are particularly disclosed herein may be administered to the lungs of a patient by any suitable means, but are preferably administered by generating an aerosol comprised of respirable particles, the respirable particles comprised of the anti-sense compound, which particles the subject inhales. The respirable particles may be liquid or solid. The particles may optionally contain other therapeutic ingredients. Particles comprised of anti-sense compound for practicing the present invention should include particles of respirable size: that is, particles of a size sufficiently small to pass through the mouth and larynx upon inhalation and into the bronchi and alveoli of the lungs. In general, particles ranging from about .5 to about 10 microns in size are respirable. Particles of non-respirable size which are included in the aerosol tend to deposit in the throat and be swallowed, and the quantity of non-respirable particles in the aerosol is preferably minimized. For nasal administration, a particle size in the range of 10-500 :m is preferred to ensure retention in the nasal cavity. Thus, particles of about 4, about 10, about 25, about 50 to about 75, about 100, about 250, about 500, and other specific ranges therewithin, are preferred. Others, however, are also contemplated within the confines of this invention.

Liquid pharmaceutical compositions of active compound for producing an aerosol can be prepared by combining the anti-sense compound with a suitable vehicle, such as sterile pyrogen free water. Other therapeutic compounds may optionally be included. Solid particulate compositions containing respirable dry particles of micronized anti-sense compound may be prepared by grinding dry anti-sense compound with a mortar and pestle, and then passing the micronized composition through a 400 mesh screen to break up or separate out large agglomerates. A solid particulate composition comprised of the anti-sense compound may optionally contain a dispersant which serves to facilitate the formation of an aerosol. A suitable dispersant is lactose, which may be blended with the anti-sense compound in any suitable ratio (e.g., a 1 to 1 ratio by weight). Again, other therapeutic compounds may also be included.

The dosage of the anti-sense compound administered will depend upon the disease being treated, the condition of the subject, the particular formulation, the route of administration, the timing

of administration to a subject, etc. In general, intracellular concentrations of the oligonucleotide of from about 0.01, about 0.05, about 0.1, about 0.2, about 1 to about 5 μM, about 50 μM, about 100 μM or more, and more particularly about 0.2 to about 0.5 μM, are desired. For administration to a subject such as a human, a dosage of from about 0.01, about 0.1 or about 1 mg/Kg up to about 50, about 100, or about 150 mg/Kg and even higher doses are typically employed depending on the route of administration as is known in the art. Depending on the solubility of the particular formulation of active compound administered, the daily dose may be divided among one or several unit dose administrations. Administration of the anti-sense compounds may be carried out therapeutically (i.e., as a rescue treatment) or prophylactically. Aerosols of liquid particles comprising the anti-sense compound may be produced by any suitable means, such as with a nebulizer. See, e.g., U.S. Patent No. 4,501,729. Nebulizers are commercially available devices which transform solutions or suspensions of the active ingredient into a therapeutic aerosol mist either by means of acceleration of a compressed gas, typically air or oxygen, through a narrow venturi orifice or by means of ultrasonic agitation. Suitable formulations for use in nebulizers consist of the active ingredient in a liquid carrier, the active ingredient comprising up to 40% w/w of the formulation, but preferably less than 20% w/w. The carrier is typically water or a dilute aqueous alcoholic solution, preferably made isotonic with body fluids by the addition of, for example, sodium chloride. Optional additives include preservatives if the formulation is not prepared sterile, for example, methyl hydroxybenzoate, antioxidants, flavoring agents, volatile oils, buffering agents and surfactants.

In one preferred embodiment, the pharmaceutical composition comprises nucleic acid(s) which comprise the anti-sense oligo(s) described above and one or more surfactants. Suitable surfactants or surfactant components for enhancing the uptake of the anti-sense oligonucleotides of the invention include synthetic and natural as well as full and truncated forms of surfactant protein A, surfactant protein B, surfactant protein C, surfactant protein D and surfactant Protein E, di-saturated phosphatidylcholine (other than dipalmitoyl), dipalmitoylphosphatidylcholine, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine; phosphatidic acid, ubiquinones, lysophosphatidylethanolamine, lysophosphatidylcholine, palmitoyl-lysophosphatidylcholine, dehydroepiandrosterone, dolichols, sulfatidic acid, glycerol-3-phosphate, dihydroxyacetone phosphate, glycerol, glycero-3-phosphocholine, dihydroxyacetone, palmitate, cytidine diphosphate (CDP) diacylglycerol, CDP choline, choline, choline phosphate; as well as natural and artificial lamellar bodies which are the natural carrier vehicles for the components of surfactant, omega-3 fatty acids, polyenic acid, polyenoic acid, lecithin, palmitinic acid, non-ionic block copolymers of ethylene or propylene oxides, polyoxypropylene, monomeric and polymeric, polyoxyethylene, monomeric and polymeric, poly (vinyl amine) with dextran and/or alkanoyl side chains, Brij 35, Triton X-100 and synthetic surfactants ALEC, Exosurf, Survan and Atovaquone, among others. These surfactants may be used either as a single, or as part of a multiple component, surfactant in a formulation, or as covalently bound additions to the 5' and/or 3' ends of the anti-sense oligo(s). Aerosols of solid particles comprising the active compound may likewise be produced with any solid particulate medicament aerosol generator. Aerosol generators for administering solid particulate medicaments to a subject produce particles which are respirable, as explained above, and generate a volume of aerosol containing a predetermined metered dose of a medicament at a rate suitable for human administration. One illustrative type of solid particulate aerosol generator is an insufflator. Suitable formulations for administration by insufflation include finely comminuted powders which may be delivered by means of an insufflator or taken into the nasal cavity in the manner of a snuff. In the insufflator, the powder (e.g., a metered dose thereof effective to carry out the treatments described herein) is contained in capsules or cartridges, typically made of gelatin or plastic, which are either pierced or opened in situ and the powder delivered by air drawn through the device upon inhalation or by means of a manually-operated pump. The powder employed in the insufflator consists either solely of the active ingredient or of a powder blend comprising the active ingredient, a suitable powder diluent, such as lactose, and an optional surfactant. The active

ingredient typically comprises from 0.1 to 100 w/w of the formulation. A second type of illustrative aerosol generator comprises a metered dose inhaler. Metered dose inhalers are pressurized aerosol dispensers, typically containing a suspension or solution formulation of the active ingredient in a liquefied propellant. During use these devices discharge the formulation through a valve adapted to deliver a metered volume, typically from 10 to 150 :l, to produce a fine particle spray containing the active ingredient. Suitable propellants include certain chlorofluorocarbon compounds, for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane and mixtures thereof. The formulation may additionally contain one or more co-solvents, for example, ethanol, surfactants, such as oleic acid or sorbitan trioleate, antioxidants and suitable flavoring agents. The aerosol, whether formed from solid or liquid particles, may be produced by the aerosol generator for example at a rate of from about 10, about 30, about 70 to about 100, about 150, about 150 liters per minute, more preferably from about 30 to 150 liters per minute, and most preferably about 60 liters per minute. Aerosols containing greater amounts of medicament, however, may be administered more rapidly as is known in the art.

The relevant disclosures of all scientific publications and patent references cited in this patent are specifically intended to be incorporated herein by reference, particularly in reference to preparatory methods and technologies which are enabling of the invention. The following examples are provided to illustrate the present invention, and should not be construed as limiting thereon.

EXAMPLES

In the following examples, :M means micromolar, ml means milliliters, :m means micrometers, mm means millimeters, cm means centimeters, EC means degrees Celsius, :g means micrograms, mg means milligrams, g means grams, kg means kilograms, M means molar, and h or hr. means hours.

Example 1: Design and Synthesis of Anti-sense Oligonucleotides

The design of anti-sense oligonucleotides against the A_1 and A_3 adenosine receptors may require the solution of the complex secondary structure of the target A_1 receptor mRNA and the target A_3 receptor mRNA. After generating this structure, anti-sense nucleotide are designed which target regions of mRNA which might be construed to confer functional activity or stability to the mRNA and which optimally may overlap the initiation codon. Other target sites are readily usable. As a demonstration of specificity of the anti-sense effect, other oligonucleotides not totally complementary to the target mRNA, but containing identical nucleotide compositions on a w/w basis, are included as controls in anti-sense experiments.

The mRNA secondary structure of the adenosine A_1 receptor was analyzed and used as described above. to design a phosphorothioate anti-sense oligonucleotide. The anti-sense oligonucleotide which was synthesized was designated HAdA$_1$AS and had the following sequence: **5' -GAT GGA GGG CGG CAT GGC GGG-3' (SEQ ID NO:1).** As a control, a mismatched phosphorothioate anti-sense nucleotide designated HAdAlMM1 was synthesized with the following sequence: **5' -GTA GCA GGC GGG GAT GGG GGC-3' (SEQ ID NO:2).** Each oligonucleotide had identical base content and general sequence structure. Homology searches in GENBANK (release 85.0) and EMBL (release 40.0) indicated that the anti-sense oligonucleotide was specific for the human and rabbit adenosine A_1 receptor genes, and that the mismatched control was not a candidate for hybridization with any known gene sequence.

The secondary structure of the adenosine A_3 receptor mRNA was similarly analyzed and used as described above to design two phosphorothioate anti-sense oligonucleotides. The first anti-sense oligonucleotide (HAdA3AS1) synthesized had the following sequence: **5' -GTT GTT GGG CAT CTT GCC-3' (SEQ ID NO:3).** As a control, a mismatched phosphorothioate anti-sense oligonucleotide (HAdA3MM1) was synthesized, having the following sequence: **5' -GTA CTT GCG GAT CTA GGC-3' (SEQ ID NO:4).** A second phosphorothioate anti-sense oligonucleotide (HAdA3AS2) was also designed and synthesized, having the following sequence: **5' -GTG GGC CTA**

GCT CTC GCC-3' (SEQ ID NO:5). Its control oligonucleotide (HAdA3MM2) had the sequence: 5' -GTC GGG GTA CCT GTC GGC-3' (SEQ ID NO:6). Phosphorothioate oligonucleotides were synthesized on an Applied Biosystems Model 396 Oligonucleotide Synthesizer, and purified using NENSORB chromatography (DuPont, MD).

Example 2: In Vivo Testing of Adenosine A_1 Receptor Anti-sense Oligos

The anti-sense oligonucleotide against the human A_1 receptor (SEQ ID NO:1) described above. was tested for efficacy in an in vitro model utilizing lung adenocarcinoma cells HTB-54. HTB-54 lung adenocarcinoma cells were demonstrated to express the A_1 adenosine receptor using standard northern blotting procedures and receptor probes designed and synthesized in the laboratory.

HTB-54 human lung adenocarcinoma cells (106/100 mm tissue culture dish) were exposed to 5.0 :M HAdAlAS or HAdAlMM1 for 24 hours, with a fresh change of media and oligonucleotides after 12 hours of incubation. Following 24 hour exposure to the oligonucleotides, cells were harvested and their RNA extracted by standard procedures. A 21-mer probe corresponding to the region of mRNA targeted by the anti-sense (and therefore having the same sequence as the anti-sense, but not phosphorothioated) was synthesized and used to probe northern blots of RNA prepared from HAdAlAS-treated, HAdAlMM1-treated and non-treated HTB-54 cells. These blots showed clearly that HAdAlAS but not HAdAlMM1 effectively reduced human adenosine receptor mRNA by >50%. This result showed that HAdAlAS is a good candidate for an anti-asthma drug since it depletes intracellular mRNA for the adenosine A_1 receptor, which is involved in asthma.

Example 3: In Vivo Efficacy of Adenosine A_1 Receptor Anti-sense Oligos

A fortuitous homology between the rabbit and human DNA sequences within the adenosine A_1 gene overlapping the initiation codon permitted the use of the phosphorothioate anti-sense oligonucleotides initially designed for use against the human adenosine A_1 receptor in a rabbit model. Neonatal New Zealand white Pasteurella-free rabbits were immunized intraperitoneally within 24 hours of birth with 312 antigen units/ml house dustmite (D. farinae) extract (Berkeley Biologicals, Berkeley, CA), mixed with 10% kaolin. Immunizations were repeated weekly for the first month and then biweekly for the next 2 months. At 3-4 months of age, eight sensitized rabbits were anesthetized and relaxed with a mixture of ketamine hydrochloride (44 mg/kg) and acepromazine maleate (0.4 mg/kg) administered intramuscularly. The rabbits were then laid supine in a comfortable position on a small molded, padded animal board and intubated with a 4.0-mm intratracheal tube (Mallinkrodt, Inc., Glens Falls, NY). A polyethylene catheter of external diameter 2.4 mm with an attached latex balloon was passed into the esophagus and maintained at the same distance (approximately 16 cm) from the mouth throughout the experiments. The intratracheal tube was attached to a heated Fleisch pneumotachograph (size 00; DOM Medical, Richmond, VA), and flow was measured using a Validyne differential pressure transducer (Model DP-45161927; Validyne Engineering Corp., Northridge, CA) driven by a Gould carrier amplifier (Model 11-4113; Gould Electronic, Cleveland, OH). The esophageal balloon was attached to one side of the differential pressure transducer, and the outflow of the intratracheal tube was connected to the opposite side of the pressure transducer to allow recording of transpulmonary pressure. Flow was integrated to give a continuous tidal volume, and measurements of total lung resistance (RL) and dynamic compliance (Cdyn) were calculated at isovolumetric and flow zero points, respectively, using an automated respiratory analyzer (Model 6; Buxco, Sharon, CT). Animals were randomized and on Day 1 pretreatment values for PC50 were obtained for aerosolized adenosine. Anti-sense (HAdAlAS) or mismatched control (HAdAlMM) oligonucleotides were dissolved in sterile physiological saline at a concentration of 5000 :g (5 mg) per 1.0 ml. Animals were subsequently administered the aerosolized anti-sense or mismatch oligonucleotide via the intratracheal tube (approximately 5000 :g in a volume of 1.0 ml), twice daily for two days. Aerosols of either saline, adenosine, or anti-sense or mismatch oligonucleotides were generated by an ultrasonic nebulizer (DeVilbiss, Somerset, PA), producing aerosol droplets 80% of which were smaller than 5 :m in

diameter. In the first arm of the experiment, four randomly selected allergic rabbits were administered anti-sense oligonucleotide and four the mismatched control oligonucleotide. On the morning of the third day, PC50 values (the concentration of aerosolized adenosine in mg/ml required to reduce the dynamic compliance of the bronchial airway 50% from the baseline value) were obtained and compared to PC50 values obtained for these animals prior to exposure to oligonucleotide. Following a 1 week interval, animals were crossed over, with those previously administered mismatch control oligonucleotide now administered anti-sense oligonucleotide, and those previously treated with anti-sense oligonucleotide now administered mismatch control oligonucleotide. Treatment methods and measurements were identical to those employed in the first arm of the experiment. It should be noted that in six of the eight animals treated with anti-sense oligonucleotide, adenosine-mediated bronchoconstriction could not be obtained up to the limit of solubility of adenosine, 20 mg/ml. For the purpose of calculation, PC50 values for these animals were set at 20 mg/ml. The values given therefore represent a minimum figure for anti-sense effectiveness. Actual effectiveness was higher. The results of this experiment are illustrated in Table 5 below.

Table 5: Effect of Adenosine A_1 Receptor Anti-sense Oligo upon PC50 Values in Asthmatic Rabbits

Mismatch Control		A_1 Receptor Anti-sense Oligo	
Pre Oligonucleotide	**Post Oligonucleotide**	**Pre Oligonucleotide**	**Post Oligonucleotide**
3.56 ± 1.02	5.16 ± 1.03	2.36 ± 0.68	>19.5 ± 0.34**

The results are presented as the mean (n=8) ± SEM.
The significance was determined by repeated-measures analysis of variance (ANOVA), and Tukey's protected test.
**Significantly different from all other groups, p<0.01.

In both arms of the experiment, animals receiving the anti-sense oligonucleotide showed an order of magnitude increase in the dose of aerosolized adenosine required to reduce dynamic compliance of the lung by 50%. No effect of the mismatched control oligonucleotide upon PC50 values was observed. No toxicity was observed in any animal receiving either anti-sense or control inhaled oligonucleotide. These results show clearly that the lung has exceptional potential as a target for anti-sense oligonucleotide-based therapeutic intervention in lung disease. They further show, in a model system which closely resembles human asthma, that downregulation of the adenosine A_1 receptor largely eliminates adenosine-mediated bronchoconstriction in asthmatic airways. Bronchial hyperresponsiveness in the allergic rabbit model of human asthma is an excellent endpoint for anti-sense intervention since the tissues involved in this response lie near to the point of contact with aerosolized oligonucleotides, and the model closely simulates an important human disease.

Example 4: Specificity of A_1-adenosine Receptor Anti-sense Oligonucleotide

At the conclusion of the cross-over experiment of Example 3 above, airway smooth muscle from all rabbits was quantitatively analyzed for adenosine A_1 receptor number. As a control for the specificity of the anti-sense oligonucleotide, adenosine A_2 receptors, which should not have been affected, were also quantified. Airway smooth muscle tissue was dissected from each rabbit and a membrane fraction prepared according to the method of Kleinstein et al. (Kleinstein, J. and Glossmann, H., Naunyn-Schmiedeberg's Arch. Pharmacol. 305: 191-200 (1978)), the relevant portion of which is hereby incorporated in its entirety by reference, with slight modifications. Crude plasma membrane preparations were stored at 70EC until the time of assay. Protein content was determined by the method of Bradford (M. Bradford, Anal. Biochem. 72, 240-254 (1976), the relevant portion of which is hereby incorporated in its entirety by reference). Frozen plasma membranes were thawed at room temperature and were incubated with 0.2 U/ml adenosine deaminase for 30 minutes at 37EC to remove endogenous adenosine. The binding of [^{3}H] DPCPX (A_1 receptor-specific) or [^{3}H] CGS-21680 (A_1 receptor-specific) was measured as previously described by Ali et al. (Ali, S. et al., J. Pharmacol. Exp. Ther. 268, Am. J. Physiol 266, L271-277 (1994), the relevant portion of which is hereby incorporated in its entirety by reference). The animals treated with adenosine A_1 anti-sense oligonucleotide in the cross-over experiment had a nearly 75% decrease in A_1 receptor number compared to controls, as assayed by specific binding of the A_1-specific antagonist DPCPX. There was

no change in adenosine A_2 receptor number, as assayed by specific binding of the A_2 receptor-specific agonist 2- [p- (2-carboxyethyl)-phenethylamino] -5' - (N-ethylcarboxamido) adenosine (CGS-21680). This is illustrated in Table 6 below.

Table 6: Specificity of Action of Adenosine A_1 Receptor Oligonucleotide Anti-sense

	Mismatch Control Oligonucleotide	A_1 Anti-sense Oligonucleotide
A_1-Specific Binding	1105 ± 48**	293 ± 18
A_2-Specific Binding	302 ± 22	442 ± 171

The results are presented as the mean (n = 8) ± SEM.
The significance was determined by repeated-measures analysis of variance (ANOVA), and Tukey's protected test.
**Significantly different from mismatch control, p<0.01.

The above results illustrate the effectiveness of anti-sense oligonucleotides in treating airway disease. Since the anti-sense oligos described above. eliminate the receptor systems responsible for adenosine-mediated bronchoconstriction, it may be less imperative to eliminate adenosine from them. However, it would be preferable to eliminate adenosine from even these oligonucleotides to reduce the dose needed to attain a similar effect. Described above are other anti-sense oligonucleotides targeting mRNA of proteins involved in inflammation. Adenosine has been eliminated from their nucleotide content to prevent its liberation during degradation.

Example 5: Anti-sense Oligos directed to other Target Nucleic Acids

This work was conducted to demonstrate that the present invention is broadly applicable to anti-sense oligonucleotides ("oligos") specific to nucleic acid targets broadly. The following experimental studies were conducted to show that the method of the invention is broadly suitable for use with anti-sense oligos designed as taught by this application and targeted to any and all adenosine receptor mRNAs. For this purpose, various anti-sense oligos were porepared to adenosine receptor mRNAs exemplified by the adenosine A_1, A_{2b} and A_3 receptor mRNAs. Anti-sense Oligo I was disclosed above (**SEQ. ID NO:1**).Five additional anti-sense phosphorothioate oligos were designed asnd synthesized as indicated above.

1- Oligo II (**SEQ. ID NO: 7**) also targeted to the adenosine A_1 receptor, but to a different region than Oligo I.
2- Oligo V (**SEQ. ID NO: 10**) targeted to the adenosine A_{2b} receptor.
3- Oligos III (**SEQ. ID NO: 8**) and IV (**SEQ. ID NO: 9**) targeted to different regions of the adenosine A_3 receptor.
4- Oligo I-PD (**SEQ. ID NO: 1681**)(a phosphodiester oligo of the same sequence as Oligo I).

These anti-sense oligos were designed for therapy on a selected species as described above and are generally specific for that species, unless the segment of the target mRNA of other species happens to contain a similar sequences. All anti-sense oligos were prepared as described below, and tested in vivo in a rabbit model for bronchoconstriction, inflammation and allergy, which have breathing difficulties and impeded lung airways, as is the case in ailments such as asthma, as described in the above-identified application.

Example 6: Design & Sequences of other Anti-sense Oligos

Six oligos and their effects in a rabbit model were studied and the results of these studies are reported and discussed below. Five of these oligos were selected for this study to complement the data on Oligo I (**SEQ ID NO: 1**) provided in Examples 1 to 4 above. This oligo is anti-sense to one region of the adenosine A_1 receptor mRNA. The oligos tested are identified as anti-sense Oligos I (**SEQ ID NO: 1**) and II (**SEQ. ID No: 7**) targeted to a different region of the adenosine A_1 receptor mRNA, Oligo V (**SEQ. ID No:8**) targeted to the adenosine A_{2b} receptor mRNA, and anti-sense Oligos III and IV (**SEQ. ID NOS: 9 and 10**) targeted to two different regions of the adenosine A_3 receptor mRNA. The sixth oligo (Oligo I-PD) is a phosphodiester version of Oligo I (**SEQ. ID NO:1**). The design and synthesis of these anti-sense oligos was performed in accordance with Example 1 above.

(I) Anti-sense Oligo I

The anti-sense oligonucleotide I referred to in Examples 1 to 4 above is targeted to the human

A_1 adenosine receptor mRNA (EPI 2010). Anti-sense oligo I is 21 nucleotide long, overlaps the initiation codon, and has the following sequence:5'-GAT GGA GGG CGG CAT GGC GGG-3'(SEQ.ID NO:1). The oligo I was previously shown to abrogate the adenosine-induced bronchoconstriction in allergic rabbits, and to reduce allergen-induced airway obstruction and bronchial hyperresponsiveness (BHR), as discussed above and shown by Nyce, J. W. & Metzger, W. J., Nature, 385:721 (1977), the relevant portions of which reference are incorporated in their entireties herein by reference.

(II) Anti-sense Oligo II

A phosphorothioate anti-sense oligo **(SEQ. ID NO:7)** was designed in accordance with the invention to target the rabbit adenosine A_1 receptor mRNA region +936 to +956 relative to the initiation codon (start site). The anti-sense oligo II is 21 nucleotide long, and has the following sequence: **5'-CTC GTC GCC GTC GCC GGC GGG-3' (SEQ. ID NO:7).**

(III) Anti-sense Oligo III

A phosphorothioate anti-sense oligo other than that provided in Example 1 above **(SEQ. ID NO:8)** was designed in accordance with the invention to target the anti-sense A_3 receptor mRNA region +3 to + 22 relative to the initiation codon start site. The anti-sense oligo III is 20 nucleotide long, and has the following sequence: **5'-GGG TGG TGC TAT TGT CGG GC-3' (SEQ. ID NO:8).**

(IV) Anti-sense Oligo IV

Yet another phosphorothioate anti-sense oligo **(SEQ. ID NO:9)** was designed in accordance with the invention to target the adenosine A_3 receptor mRNA region + 386 to + 401 relative to the initiation codon (start site). The anti-sense oligo IV is 15 nucleotide long, and has the following sequence: **5'-GGC CCA GGG CCA GCC-3' (SEQ. ID NO:9)**

(V) Anti-sense Oligo V

A phosphorothioate anti-sense oligo **(SEQ. ID NO:10)** was designed in accordance with the invention to target the adenosine A_{2b} receptor mRNA region -21 to -1 relative to the initiation codon (start site). The anti-sense oligonucleotide V is 21 nucleotide long, and has the following sequence: **5'-GGC CGG GCC AGC CGG GCC CGG-3' (SEQ. ID NO:10).**

(VI) A_1 Mismatch Oligos

Two different mismatched oligonucleotides having the following sequences were used as controls for anti-sense oligo I**(SEQ. ID NO: 1)** described in Example 5 above: A_1 MM2:**5'-GTA GGT GGC GGG CAA GGC GGG-3' (SEQ. ID NO:2421), and** A_1 MM3:**5'-GAT GGA GGC GGG CAT GGC GGG-3' (SEQ. ID NO:2422).** Anti-sense oligo I and the two mismatch anti-sense oligos had identical base content and general sequence structure. Homology searches in GENBANK (release 85.0) and EMBL (release 40.0) indicated that the anti-sense oligo I was specific, not only for the human, but also for the rabbit, adenosine A_1 receptor genes, and that the mismatched controls were not candidates for hybridization with any known human or animal gene sequence.

(VII) Anti-sense Oligo A_1-PD (Oligo VI)

A phosphodiester anti-sense oligo **(Oligo VI; SEQ. ID NO:2420)** having the same nucleotide sequence as Oligo I was designed as disclosed in the above-identified application. Anti-sense oligo I-PD is 21 nucleotide long, overlaps the initiation codon, and has the following sequence: 5'- GAT GGA GGG CGG CAT GGC GGG-3' **(SEQ. ID NO:2420).**

III) Controls

Each rabbit was administered 5.0 ml aerosolized sterile saline following the same schedule as for the anti-sense oligos in (II), (III), and (IV) above.

Example 7: Synthesis of Anti-sense Oligos

Phosphorothioate anti-sense oligos having the sequences described in (a) above, were synthesized on an Applied Biosystems Model 396 Oligonucleotide Synthesizer, and purified using NENSORB chromatography (DuPont, DE). TETD (tetraethylthiuram disulfide) was used as the sulfurizing agent during the synthesis. Anti-sense oligonucleotide II **(SEQ. ID NO:7)**, anti-sense oligonucleotide III **(SEQ. ID NO: 8)** and anti-sense oligonucleotide IV **(SEQ. ID NO: 9)** were each synthesized and purified in this manner.

Example 8: Preparation of Allergic Rabbits

Neonatal New Zealand white Pasturella-free rabbits were immunized intraperitoneally within 24 hours of birth with 0.5 ml of 312 antigen units/ml house dust mite (D. *farinae*) extract (Berkeley Biologicals, Berkeley, CA) mixed with 10% kaolin as previously described (Metzger, W. J., in Late Phase Allergic Reactions, Dorsch, W., Ed., CRC Handbook, pp. 347-362, CRC Press, Boca Raton (1990); Ali, S., Metzger, W. J. and Mustafa, S. J., Am. J. Resp. Crit. Care Med. 149: 908 (1994)), the relevant portions of which are incorporated in their entireties here by reference. Immunizations were repeated weekly for the first month and then biweekly until the age of 4 months. These rabbits preferentially produce allergen-specific IgE antibody, typically respond to aeroallergen challenge with both an early and late-phase asthmatic response, and show bronchial hyper responsiveness (BHR). Monthly intraperitoneal administration of allergen (312 units dust mite allergen, as above) continues to stimulate and maintain allergen-specific IgE antibody and BHR. At 4 months of age, sensitized rabbits were prepared for aerosol administration as described by Ali et al. (Ali, S., Metzger, W. J. and Mustafa, S. J., Am. J. Resp. Crit. Care Med. 149 (1994)), the relevant section being incorporated in its entirety here by reference.

DOSE-RESPONSE STUDIES

Example 9: Experimental Setup

Aerosols of either adenosine (0-20 mg/ml), or anti-sense or one of two mismatch oligonucleotides (5 mg/ml) were separately prepared with an ultrasonic nebulizer (Model 646, DeVilbiss, Somerset, PA), which produced aerosol droplets, 80% of which were smaller than 5:m in diameter. Equal volumes of the aerosols were administered directly to the lungs *via* an intratracheal tube. The animals were randomized, and administered aerosolized adenosine. Day 1 pre-treatment values for sensitivity to adenosine were calculated as the dose of adenosine causing a 50% loss of compliance (PC_{50} Adenosine). The animals were then administered either the aerosolized anti-sense or one of the mismatch anti-sense oligos via the intratracheal tube (5 mg/1.0 ml), for 2 minutes, twice daily for 2 days (total dose, 20 mg). Post-treatment PC_{50} values were recorded (post-treatment challenge) on the morning of the third day. The results of these studies are provided in Example 21 below.

Example 10: Crossover Experiments

For some experiments utilizing anti-sense oligo I (**SEQ ID NO: 1**) and a corresponding mismatch control oligonucleotide A1MM2, following a 2 week interval, the animals were crossed over, with those previously administered the mismatch control A_1MM2, now receiving the anti-sense oligo I, and those previously treated with the anti-sense oligo I, now receiving the mismatch control A_1MM2 oligo. The number of animals per group was as follows. For mismatch A_1MM2 (Control 1), n=7, since one animal was lost in the second control arm of the experiment due to technical difficulties, for mismatch A_1MM3 n=4 (Control 2) and for A_1AS anti-sense oligo I, n=8. The A_1MM3 oligo-treated animals were analyzed separately and were not part of the cross-over experiment. The treatment methods and measurements employed following the cross-over were identical to those employed in the first arm of the experiment. In 6 of the 8 animals treated with the anti-sense oligo I (**SEQ. ID NO: 1**), no PC_{50} value could be obtained for adenosine doses of up to 20 mg/ml, which is the limit of solubility of adenosine. Accordingly, the PC_{50} values for these animals were assumed to be 20 mg/ml for calculation purposes. The values given, therefore, represent a minimum figure for the effectiveness of the anti-sense oligonucleotides of the invention. Other groups of allergic rabbits (n=4 for each group) were administered 0.5 or 0.05 mg doses of the anti-sense oligo I (**SEQ ID NO: 1**), or the A_1MM2 oligo in the manner and according to the schedule described above (the total doses being 2.0 or 0.2 mg). The results of these studies are provided in Example 22 below.

Example 11: Anti-sense Oligo Formulation

Each one of anti-sense oligos were separately solubilized in an aqueous solution and

administered as described for anti-sense oligo I (**SEQ. ID No:1**) in (e) above, in four 5 mg aliquots (20 mg total dose) by means of a nebulizer via endotracheal tube, as described above. The results obtained for anti-sense oligo I and its mismatch controls confirmed that the mismatch controls are equivalent to saline, as described in Example 19 below and in Table 1 of Nyce & Metzger, Nature 385: 721-725 (1997). Because of this finding, saline was used as a control for pulmonary function studies employing anti-sense oligos II, III and IV (**SEQ. IS NOS; 7, 8 and 9**).

Example 12: Specificity of Oligo I for Adenosine A_1 Receptor (Receptor Binding Studies)

Tissue from airway smooth muscle was dissected to primary, secondary and tertiary bronchi from rabbits which had been administered 20 mg oligo I (**SEQ ID NO: 1**) in 4 divided doses over a period of 48 hours as described above. A membrane fraction was prepared according to the method of Ali et al. (Ali, S., et al., Am. J. Resp. Crit. Care Med. 149: 908 (1994), the relevant section relating to the preparation of the membrane fraction is incorporated in its entirety hereby by reference). The protein content was determined by the method of Bradford and plasma membranes were incubated with 0.2 U/ml adenosine deaminase for 30 minutes at 37EC to remove endogenous adenosine. See, Bradford, M. M. Anal. Biochem. 72, 240-254 (1976), the relevant portion of which is hereby incorporated in its entirety by reference. The binding of [^{3}H]DPCPX, [^{3}H]NPC17731, or [^{3}H]CGS-21680 was measured as described by Jarvis et al. See, Jarvis, M.F., et al., Pharmacol. Exptl. Ther. 251, 888-893 (1989), the relevant portion of which is fully incorporated herein by reference. The results of this study are shown in Table 8 and discussed in Example 20 below.

Example 13: Pulmonary Function Measurements (Compliance c_{DYN} and Resistance)

At 4 months of age, the immunized animals were anesthetized and relaxed with 1.5 ml of a mixture of ketamine HCl (35 mg/kg) and acepromazine maleate (1.5 mg/kg) administered intramuscularly. After induction of anesthesia, allergic rabbits were comfortably positioned supine on a soft molded animal board. Salve was applied to the eyes to prevent drying, and they were closed. The animals were then intubated with a 4.0 mm intermediate high-low cuffed Murphy 1 endotracheal tube (Mallinckrodt, Glen Falls, NY), as previously described by Zavala and Rhodes. See, Zavala and Rhodes, Proc. Soc. Exp. Biol. Med. 144: 509-512 (1973), the relevant portion of which is incorporated herein by reference in its entirety. A polyethylene catheter of OD 2.4 mm (Becton Dickinson, Clay Adams, Parsippany NJ) with an attached thin-walled latex balloon was passed into the esophagus and maintained at the same distance (approximately 16 cm) from the mouth throughout the experiment. The endotracheal tube was attached to a heated Fleisch pneumotach (size 00; DEM Medical, Richmond, VA), and the flow (v) measured using a Validyne differential pressure transducer (Model DP-45-16-1927, Validyne Engineering, Northridge, CA), driven by a Gould carrier amplifier (Model 11-4113, Gould Electronics, Cleveland, OH). An esophageal balloon was attached to one side of the Validyne differential pressure transducer, and the other side was attached to the outflow of the endotracheal tube to obtain transpulmonary pressure (P_{tp}). The flow was integrated to yield a continuous tidal volume, and the measurements of total lung resistance (R_t) and dynamic compliance (C_{dyn}) were made at isovolumetric and zero flow points. The flow, volume and pressure were recorded on an eight channel Gould 2000 W high-frequency recorder and C_{dyn} was calculated using the total volume and the difference in P_{tp} at zero flow, and . R_t was calculated as the ratio of Ptp and V at midtidal lung volumes. These calculations were made automatically with the Buxco automated pulmonary mechanics respiratory analyzer (Model 6, Buxco Electronics, Sharon, CT), as previously described by Giles et al. See, Giles et al., Arch. Int. Pharmacodyn. Ther. 194: 213-232 (1971), the relevant portion of which describing these calculations is incorporated in toto hereby by reference. The results obtained upon administration of oligo II on allergic rabbits are shown and discussed in Example 26 below.

Example 14: Measurement of Bronchial Hyperresponsiveness (BHR)

Each allergic rabbit was administered histamine by aerosol to determine their baseline

hyperresponsiveness. Aerosols of either saline or histamine were generated using a DeVilbiss nebulizer (DeVilbiss, Somerset, PA) for 30 seconds and then for 2 minutes at each dose employed. The ultrasonic nebulizer produced aerosol droplets of which 80% were <5 micron in diameter. The histamine aerosol was administered in increasing concentrations (0.156 to 80 mg/ml) and measurements of pulmonary function were made after each dose. The B4R was then determined by calculating the concentration of histamine (mg/ml) required to reduce the C_{dyn} 50% from baseline ($PC_{50\ Histamine}$).

Example 15: Cardiovascular Effect of Anti-sense Oligo I

The measurement of cardiac output and other cardiovascular parameters using CardiomaxJ utilizes the principal of thermal dilution in which the change in temperature of the blood exiting the heart after a venous injection of a known volume of cool saline is monitored. A single rapid injection of cool saline was made into the right atrium via cannulation of the right jugular vein, and the corresponding changes in temperature of the mixed injectate and blood in the aortic arch were recorded via cannulation of the carotid artery by a temperature-sensing miniprobe. Twelve hours after the allergic rabbits had been treated with aerosols of oligo I (EPI 2010; SEQ. ID NO: 1) as described in (d) above, the animals were anesthetized with 0.3 ml/kg of 80% Ketamine and 20% Xylazine. This time point coincides with previous data showing efficacy for SEQ. ID NO: 1, as is clearly shown by Nyce & Metzger, (1997), supra, the pertinent disclosure being incorporated in its entirety here by reference. A thermocouple was then inserted into the left carotid artery of each rabbit, and was then advanced 6.5 cm and secured with a silk ligature. The right jugular vein was then cannulated and a length of polyethylene tubing was inserted and secured. A thermodilution curve was then established on a CardiomaxJ II (Columbus Instruments, Ohio) by injecting sterile saline at 20EC to determine the correctness of positioning of the thermocouple probe. After establishing the correctness of the position of the thermocouple, the femoral artery and vein were isolated. The femoral vein was used as a portal for drug injections, and the femoral artery for blood pressure and heart rate measurements. Once constant baseline cardiovascular parameters were established, CardiomaxJ measurements of blood pressure, heart rate, cardiac output, total peripheral resistance, and cardiac contractility were made.

Example 16: Duration of Action of Oligo I (SEQ. ID NO: 1)

Eight allergic rabbits received initially increasing log doses of adenosine by means of a nebulizer via an intra-tracheal tube as described in (f) above, beginning with 0.156 mg/ml until compliance was reduced by 50% ($PC_{50\ Adenosine}$) to establish a baseline. Six of the rabbits then received four 5 mg aerosolized doses of (SEQ. ID NO: 1) as described above. Two rabbits received equivalent amounts of saline vehicle as controls. Beginning 18 hours after the last treatment, the $PC_{50\ Adenosine}$ values were tested again. After this point, the measurements were continued for all animals each day, for up to 10 days. The results of this study are discussed in Example 25 below.

Example 17: Reduction of Adenosine A_{2b} Receptor Number by Anti-sense Oligo V

Sprague Dawley rats were administered 2.0 mg respirable anti-sense oligo V (SEQ ID NO:10) three times over two days using an inhalation chamber as described above. Twelve hours after the last administration, lung parenchymal tissue was dissected and assayed for adenosine A_{2b} receptor binding using [311]-NECA as described by Nyce & Metzger (1997), supra. Controls were conducted by administration of equal volumes of saline. The results are significant at $p<0.05$ using Student's paired t test, and are discussed in Example 28 below.

Example 18: Comparison of Oligo I & Corresponding Phosphodiester Oligo VI (SEQ. ID NO:1681)

Oligo I (SEQ ID NO:1) countered the effects of adenosine and eliminated sensitivity to it for adenosine amounte up to 20 mg adenosine/5.0 ml (the limit of solubility of adenosine). Oligo VI (SEQ ID NO:1681), the phosphodiester version of the oligonucleotide sequence, was completely ineffective when tested in the same manner. Both compounds have identical sequence, differing only in the

presence of phosphorothioate residues in Oligo I (SEQ ID NO:1), and were delivered as an aerosol as described above and in Nyce & Metzger (1997), supra. Significantly different at $p<0.001$, Student's paired t test. The results are discussed in Example 29 below.

RESULTS OBTAINED FOR ANTI-SENSE OLIGO I (SEQ. ID NO: 1)

Example 19: Results of Prior Work

The nucleotide sequence and other data for anti-sense oligo I (SEQ. ID NO: 1), which is specific for the adenosine A_1 receptor, were provided above. The experimental data showing the effectiveness of oligo I in down regulating the receptor number and activity were also provided above. Further information on the characteristics and activities of anti-sense oligo I is provided in Nyce, J. W. and Metzger, W. J., Nature 385:721 (1997), the relevant parts of which relating to the following results are incorporated in their entireties herein by reference. The Nyce & Metzger (1997) publication provided data showing that the anti-sense oligo I (SEQ. ID NO: 1):

(1) The anti-sense oligo I reduces the number of adenosine A_1 receptors in the bronchial smooth muscle of allergic rabbits in a dose-dependent manner as may be seen in Table 5 below.

(2) Anti-sense Oligo I attenuates adenosine-induced bronchoconstriction and allergen-induced bronchoconstriction.

(3) The Oligo I attenuates bronchial hyperresponsiveness as measured by PC_{50} histamine, a standard measurement to assess bronchial hyperresponsiveness. This result clearly demonstrates anti-inflammatory activity of the anti-sense oligo I as is shown in Table 5 above.

(4) As expected, because it was designed to target it, the anti-sense oligo I is totally specific for the adenosine A_1 receptor, and has no effect at all at any dose on either the very closely related adenosine A_2 receptor or the related bradykinin B_2 receptor. This is seen in Table 5 below.

(5) In contradistinction to the above effects of the Oligo I, the mismatch control molecules MM2 and MM3 (SEQ. ID NO:1682 and SEQ. ID NO:1683) which have identical base composition and molecular weight but differed from the anti-sense oligo I (SEQ ID NO: 1) by 6 and 2 mismatches, respectively. These mismatches, which are the minimum possible while still retaining identical base composition, produced absolutely no effect upon any of the targeted receptors (A_1, A_2 or B_2).

These results, along with a complete lack of prior art on the use of anti-sense oligonucleotides, such as oligo I, targeted to the adenosine A_1 receptor, are unexpected results. The showings presented in this patent clearly enable and demonstrate the effectiveness, for their intended use, of the claimed agents and method for treating a disease or condition associated with lung airway, such as bronchoconstriction, inflammation, allergy(ies), and the like.

Example 20: Oligo I Significantly Reduces Response to Adenosine Challenge

The receptor binding experiment is described in Example 12 above, and the results shown in Table 5 below which shows the binding characteristics of the adenosine A_1-selective ligand [$_3$H]DPCPX and the bradykinin B_2-selective ligand [^{3}H]NPC 17731 in membranes isolated from airway smooth muscle of A_1 adenosine receptor and B_2 bradykinin receptor anti-sense- and mismatch-treated allergic rabbits.

Table 5: Binding Characteristics of Three Anti-Sense Oligos

Treatment[1]	A_1 receptor		B_2 receptor	
	Kd	B_{max}	Kd	Bmax
Adenosine A_1	**Receptor**			
20 mg	0.36±0.029 nM	19±1.52 fmoles*	0.39±0.031 nM	14.8±0.99fmoles

2 mg	0.38±0.030 nM	32±2.56 fmoles*	0.41±0.028 nM	15.5±1.08 fmoles
0.2 mg	0.37±0.030 nM	49±3.43 fmoles	0.34±0.024 nM	19.0±1.06 fmoles
A_1MM1	**(Control)**			
20 mg	0.34±0.027 nM	52.0±3.64 fmoles	0.35±0.024 nM	14.0±1.0 fmoles
2 mg	0.37±0.033 nM	51.8±3.88 fmoles	0.38±0.028 nM	14.6±1.02 fmoles
B_2A (Bradykinin	**Receptor)**			
20 mg	0.36±0.028 nM	45.0±3.15 fmoles	0.38±0.027 nM	8.7±0.62 fmoles*
2 mg	0.39±0.035 nM	44.3±2.90 fmoles	0.34±0.024 nM	19.0±1.76 fmoles**
0.2 mg	0.40±0.028 nM	47.0±3.76 fmoles	0.35±0.028 nM	15.1±1.05 fmoles
B_2MM				
20 mg	**(Control)** 0.39±0.031 nM	42.0±2.94 fmoles	0.41±0.029 nM	14.0±0.98 fmoles
2 mg	0.41±0.035 nM	40.0±3.20 fmoles	0.37±0.030 nM	14.8±0.99 fmoles
0.2 mg	0.37±0.029 nM	43.0±3.14 fmoles	0.36±0.025 nM	15.1±1.35 fmoles
Saline Control	0.37±0.041	46.0±5.21	0.39±0.047 nM	14.2±1.35 fmoles

[1] Refers to total oligo administered in four equivalently divided doses over a 48 hour period. Treatments and analyses were performed as described in methods. Significance was determined by repeated-measures analysis of variance (ANOVA), and Tukey=s protected t test. n = 4-6 for all groups.
* Significantly different from mismatch control- and saline-treated groups, p<0.001;
**Significantly different from mismatch control- and saline-treated groups, p<0.05.

Example 21: Dose-response Effect of Oligo I

Anti-sense oligo I (**SEQ ID NO:1**) was found to reduce the effect of adenosine administration to the animal in a dose-dependent manner over the dose range tested as shown in Table 6 below.

Table 6: Dose-Response Effect to Anti-sense Oligo I

Total Dose (mg)	**$PC_{50\ Adenosine}$ (mg Adenosine)**
Anti-sense Oligo I	
0.2	8.32±7.2
2.0	14.0±7.2
20	19.5±0.34
A_1MM2 oligo (control)	
0.2	2.51±0.46
2.0	3.13± 0.71
20	3.25± 0.34

The above results were studied with the Student's paired t test and found to be statistically different, p=0.05

The oligo I (**SEQ. ID NO:1**), an anti-adenosine A_1 receptor oligo, acts specifically on the adenosine A_1 receptor, but not on the adenosine A_2 receptors. These results stem from the treatment of rabbits with anti-sense oligo I (**SEQ. ID NO.1**) or mismatch control oligo (**SEQ. ID NO:1682**; A_1MM2) as described in Example 9 above and in Nyce & Metzger (1997), supra (four doses of 5 mg spaced 8 to 12 hours apart via nebulizer via endotracheal tube), bronchial smooth muscle tissue excised and the number of adenosine A_1 and adenosine A_2 receptors determined as reported in Nyce & Metzger (1997), supra.

Example 22: Specificity of Oligo I (SEQ. ID NO:1) for Target Gene Product

Oligo I (SEQ. ID No:1) is specific for the adenosine A_1 receptor whereas its mismatch controls had no activity. Figure 1 depicts the results obtained from the cross-over experiment described in Example 10 above and in Nyce & Metzger (1997), supra. The two mismatch controls (**SEQ. ID NO:1682** and **SEQ. ID NO:1683**) evidenced no effect on the $PC_{50\ Adenosine}$ value. On the contrary, the administration of anti-sense oligo I (**SEQ. ID NO:1**) showed a seven-fold increase in the $PC_{50\ Adenosine}$

value. The results clearly indicate that the anti-sense oligo I (**SEQ. ID NO: 1**) reduces the response (attenuates the sensitivity) to exogenously administered adenosine when compared with a saline control. The results provided in Table 6 above clearly establish that the effect of the anti-sense oligo I is dose dependent (see, column 3 of Table 5). The Oligo I was also shown to be totally specific for the adenosine A_1 receptor, (see, top 3 rows of Table), inducing no activity at either the closely related adenosine A_2 receptor or the bradykinin B_2 receptor (see, lines 8-10 of Table 6 above). In addition, the results shown in Table 6 establish that the anti-sense oligo I (**SEQ. ID NO:1**) decreases sensitivity to adenosine in a dose dependent manner, and that it does this in an anti-sense oligo-dependent manner since neither of two mismatch control oligonucleotides (A_1MM2; **SEQ. ID NO:1682** and A_1MM3; **SEQ. ID NO:1683**) show any effect on $PC_{50\ Adenosine}$ values or on attenuating the number of adenosine A_1 receptors.

Example 23: Effect on Aeroallergen-induced Bronchoconstriction & Inflammation

The Oligo I (**SEQ. ID NO:1**) was shown to significantly reduce the histamine-induced effect in the rabbit model when compared to the mismatch oligos. The effect of the anti-sense Oligo I (SEQ. ID No:1) and the mismatch oligos (A_1MM2, **SEQ. ID NO:1682** and A_1MM3, **SEQ. ID NO:1682**) on allergen-induced airway obstruction and bronchial hyperresponsiveness was assessed in allergic rabbits. The effect of the anti-sense oligo I (**SEQ. ID NO:1**) on allergen-induced airway obstruction was assessed. As calculated from the area under the plotted curve, the anti-sense oligo I significantly inhibited allergen-induced airway obstruction when compared with the mismatched control (55%, $p<0.05$; repeated measures ANOVA, and Tukey's t test). A complete lack of effect was induced by the mismatch oligo A_1MM2 (Control) on allergen induced airway obstruction. The effect of the anti-sense oligo I (**SEQ. ID NO:1**) on allergen-induced BHR was determined as above. As calculated from the $PC_{50\ Histamine}$ value , the anti-sense oligo I (**SEQ. ID NO:1**) significantly inhibited allergen-induced BHR in allergic rabbits when compared to the mismatched control (61%, $p<0.05$; repeated measures ANOVA, Tukey's t test). A complete lack of effect of the A_1MM mismatch control on allergen-induced BHR was observed. The results indicated that anti-sense oligo I (**SEQ. ID NO: 1**) is effective to protect against aeroallergen-induced bronchoconstriction (house dust mite). In addition, the anti-sense oligo I (**SEQ. ID NO:1**) was also found to be a potent inhibitor of dust mite-induced bronchial hyper responsiveness, as shown by its effects upon histamine sensitivity which indicates anti-inflammatory activity for anti-sense oligo I (**SEQ. ID NO:1**).

Example 24: Anti-sense Oligo I is Free of Deleterious Side Effects

The Oligo I (**SEQ. ID NO:1**) was shown to be free of side effects that might be toxic to the recipient. No changes in arterial blood pressure, cardiac output, stroke volume, heart rate, total peripheral resistance or heart contractility (dPdT) were observed following administration of 2.0 or 20 mg oligo I (**SEQ. ID NO:1**). The addition, the results of the measurement of cardiac output (CO), stroke volume (SV), mean arterial pressure (MAP), heart rate (HR), total peripheral resistance (TPR), and contractility (dPdT) with a CardiomaxJ apparatus (Columbus Instruments, Ohio) were assessed. These results evidenced that oligo I (**SEQ. ID NO:1**) has no detrimental effect upon critical cardiovascular parameters. More particularly, this oligo does not cause hypotension. This finding is of particular importance because other phosphorothioate anti-sense oligonucleotides have been shown in the past to induce hypotension in some model systems. Furthermore, the adenosine A_1 receptor plays an important role in sinoatrial conduction within the heart. Attenuation of the adenosine A_1 receptor by anti-sense oligo I (**SEQ. ID NO:1**) might be expected to result, therefore, in deleterious extrapulmonary activity in response to the downregulation of the receptor. This is not the case. The anti-sense oligo I (**SEQ. ID NO:1**) does not produce any deleterious intrapulmonary effects and renders the administration of the low doses of the present anti-sense oligo free of unexpected, undesirable side effects. This demonstrates that when oligo I (**SEQ. ID NO:1**) is administered directly to the lung, it does not reach the heart in significant quantities to cause deleterious effects. This is in

contrast to traditional adenosine receptor antagonists like theophylline which do escape the lung and can cause deleterious, even life-threatening effects outside the lung.

Example 25: Long Lasting Effect of Oligo I

The Oligo I (**SEQ. ID NO:1**) evidenced a long lasting effect as evidenced by the PC_{50} and Resistance values obtained upon its administration prior to adenosine challenge. The duration of the effect was measured for with respect to the PC_{50} of adenosine anti-sense oligo I when administered in four equal doses of 5 mg each by means of a nebulizer via an endotracheal tube, as described above. The effect of the agent is significant over days 1 to 8 after administration. When the effect of the anti-sense oligo I (**SEQ. ID NO:1**) had disappeared, the animals were administered saline aerosols (controls), and the $PC_{50\ Adenosine}$ values for all animals were measured again. Saline-treated animals showed base line PC_{50} adenosine values (n=6). The duration of the effect (with respect to Resistance) was measured for six allergic rabbits which were administered 20 mg of anti-sense oligo I (**SEQ. ID NO: 1**) as described above, upon airway resistance measured as also described above. The mean calculated duration of effect was 8.3 days for both PC_{50} adenosine ($p<0.05$) and resistance ($p<0.05$). These results show that anti-sense oligo I (**SEQ. ID NO:1**) has an extremely long duration of action, which is completely unexpected.

Example 26: Anti-sense Oligo II

Anti-sense oligo II, targeted to a different region of the adenosine A_1 receptor mRNA, was found to be highly active against the adenosine A_1-mediated effects. The experiment measured the effect of the administration of anti-sense oligo II (**SEQ. ID NO:7**) upon compliance and resistance values when 20 mg anti-sense oligo II or saline (control) were administered to two groups of allergic rabbits as described above. Compliance and resistance values were measured following an administration of adenosine or saline as described above in Example 13. The effect of the anti-sense oligo of the invention was different from the control in a statistically significant manner, $p<0.05$ using paired t-test, compliance; $p<0.01$ for resistance. The results showed that anti-sense oligo II (**SEQ. ID NO:7**), which targets the adenosine A_1 receptor, effectively maintains compliance and reduces resistance upon adenosine challenge.

Example 27: Antisense Oligos III and IV

Oligos III (**SEQ. ID NO:8**) and IV (**SEQ. ID NO:9**) were shown to be in fact specifically targeted to the adenosine A_3 receptor by their effect on reducing inflammation and the number of inflammatory cells present upon separate administration of 20 mg of the anti-sense oligos III (**SEQ. ID NO:8**) and IV (**SEQ. ID NO:9**) to allergic rabbits as described above. The number of inflammatory cells was determined in their bronchial lavage fluid 3 hours later by counting at least 100 viable cells per lavage. The effect of anti-sense oligos III (**SEQ. ID NO:8**) and IV (**SEQ. ID NO:9**) upon granulocytes, and upon total cells in bronchial lavage were assessed following exposure to dust mite allergen. The results showed that the anti-sense oligo IV (**SEQ. ID NO:9**) and anti-sense oligo III (**SEQ. ID NO:8**) are very potent anti-inflammatory agents in the asthmatic lung following exposure to dust mite allergen. As is known in the art, granulocytes, especially eosinophils, are the primary inflammatory cells of asthma, and the administration of anti-sense oligos III (**SEQ. ID NO:8**) and IV (**SEQ. ID NO:9**) reduced their numbers by 40% and 66%, respectively. Furthermore, anti-sense oligos IV (**SEQ. ID NO:9**) and III (**SEQ. ID NO:8**) also reduced the total number of cells in the bronchial lavage fluid by 40% and 80%, respectively. This is also an important indicator of anti-inflammatory activity by the present anti-adenosine A_3 agents of the invention. Inflammation is known to underlie bronchial hyperresponsiveness and allergen-induced bronchoconstriction in asthma. Both anti-sense oligonucleotides III (**SEQ. ID NO:8**) and IV (**SEQ. ID NO:9**), which are targeted to the adenosine A_3 receptor, are representative of an important new class of anti-inflammatory agents which may be designed to specifically target the lung receptors of each species.

Example 28: Anti-sense Oligo V

The anti-sense oligo V (**SEQ. ID NO:10**) , targeted to the adenosine A_{2b} adenosine receptor mRNA was shown to be highly effective at countering adenosine A_{2b}-mediated effects and at reducing the number of adenosine A_{2b} receptors present to less than half.

Example 29: Unexpected Superiority of Substituted over Phosphodiester-residue Oligo I-DS (SEQ. ID NO:1681)

Oligos I (**SEQ. ID NO:1**) and I-DS (**SEQ. ID NO:1681**) were separately administered to allergic rabbits as described above, and the rabbits were then challenged with adenosine. The phosphodiester oligo I-DS (**SEQ. ID NO:1681**) was statistically significantly less effective in countering the effect of adenosine whereas oligo I (**SEQ. ID NO:1**) showed high effectiveness, evidencing a $PC_{50\ Adenosine}$ of 20 mg.

Example 30: Anti-sense Oligo VI

For the present work, I designed an additional anti-sense phosphorothioate oligo targeted to the adenosine A_1 receptor (Oligo VI). This anti-sense oligo was designed for therapy on a selected species as described in the above patent application and is generally specific for that species, unless the segment of the adenosine receptor mRNA of other species elected happens to have a similar sequence. The anti-sense oligos were prepared as described below, and tested in vivo in a rabbit model for bronchoconstriction, inflammation and lung allergy, which have breathing difficulties and impeded lung airways, as is the case in ailments such as asthma, as described in the above-identified application. One additional oligo and its effect in a rabbit model was studied and the results of the study are reported and discussed below. The present oligo (anti-sense oligo VI) was selected for this study to complement the data on SEQ ID NO: 1 (Oligo I), which is anti-sense to the adenosine A_1 receptor mRNA provided in the above-identified patent application. This additional oligo is identified as anti-sense Oligo VI, and is targeted to a different region of the adenosine A_1 receptor mRNA than Oligo I. The design and synthesis of this anti-sense oligo was performed in accordance with the teaching, particularly Example 1, of the above-identified patent application. The anti-sense Oligo VI is a phosphorothioate designed to target the coding region of the rabbit adenosine A_1 receptor mRNA region +964 to +984 relative to the initiation codon (start site). The Oligo VI was prepared as described in the above-indicated application, and is 20 nucleotides long. The OligoVI is directed to the adenosine A_1 receptor gene, and has the following sequence: **5'-CGC CGG CGG GTG CGG GCC GG-3' (SEQ. ID NO:_)**. The phosphorothioate anti-sense Oligo VI having the sequence described in (5) above, was synthesized on an Applied Biosystems Model 396 Oligonucleotide Synthesizer, and purified using NENSORB chromatography (DuPont, DE). TETD (tetraethylthiuram disulfide) was used as the sulfurizing agent during the synthesis.

Example 31: Preparation of Allergic Rabbits

Neonatal New Zealand white Pasturella-free rabbits were immunized intraperitoneally within 24 hours of birth with 0.5 ml of 312 antigen units/ml house dust mite (D. farinae) extract (Berkeley Biologicals, Berkeley, CA) mixed with 10% kaolin as previously described (Metzger, W. J., in Late Phase Allergic Reactions, Dorsch, W., Ed., CRC Handbook, pp 347-362, CRC Press, Boca Raton, 1990; Ali, S. Et al., Am. J. Resp. Crit. Care Med. 149: 908 (1994)). The immunizations were repeated weekly for the first month and then bi-weekly until the animals were 4 months old. These rabbits preferentially produce allergen-specific IgE antibody, typically respond to aeroallergen challenge with both an early and late-phase asthmatic response, and show bronchial hyper responsiveness (BHR). Monthly intraperitoneal administration of allergen (312 units dust mite allergen, as above) continues to stimulate and maintain allergen-specific IgE antibody and BHR. At 4 months of age, sensitized rabbits were prepared for aerosol administration as described by Ali et al. (1994), supra.

Example 32: Adenosine Acrosol Preparation

An adenosine aerosol (20 mg/ml) was prepared with an ultrasonic nebulizer (Model 646, DeVilbiss, Somerset, PA), which produced aerosol droplets, 80% of which were smaller than 5:m in

diameter. Equal volumes of the aerosols were administered directly to the lungs via an intratracheal tube to all three rabbits. The animals were then administered the aerosolized adenosine and Day 1 pre-treatment values for sensitivity to adenosine were calculated as the dose of adenosine causing a 50% loss of compliance (PC_{50} Adenosine). The animals were then administered the aerosolized anti-sense via the intratracheal tube (5 mg/1.0 ml), for 2 minutes, twice daily for 2 days (total dose, 20 mg). Post-treatment PC_{50} values were recorded (post-treatment challenge) on the morning of the third day. The results of these studies are provided in (9) below.

Example 33: Anti-sense Oligo Formulation

Each one of anti-sense oligos were separately solubilized in an aqueous solution and administered as described for anti-sense oligo I in (e) above, in four 5 mg aliquots (20 mg total dose) by means of a nebulizer via endotracheal tube, as described above.

Example 34: Oligo VI Reduces Response to Adenosine Challenge as well or Better than Oligo I

Oligo VI was tested in three allergic rabbits of the characteristics and readied as described in (7) above and in the above-indicated patent application. Oligo VI targets a section of the coding region of the A_1 receptor which is different from Oligo I. Both these target sequences were selected randomly from many possible coding region target sequences. The three rabbits were treated identically as previously indicated for Oligo I. Briefly, 5 mg of Oligo VI were nebulized to the rabbits twice per day at 8 hour intervals, for two days. Thereafter, PC_{50} adenosine studies were performed on the morning of the third day and compared to pre-treatment PC_{50} values. This protocol is described in more detail in Nyce and Metzger (Nyce & Metzger, Nature 385: 721-725 (1997)). The results obtained for the three rabbits are shown in Table 7 below.

Table 7: PC_{50} Adenosine before & after Aerosolized Adenosine Treatment

Treatment Time	PC_{50} Adenosine (mg)
Pre-treatment	3.0 ±2.1
Post-treatment	>20.0*

* maximum achievable dose due to adenosine insolubility in saline

All three animals treated with Oligo VI completely eliminated sensitivity to adenosine up to the measurable level of the agent shown in Table 7 above. That is, the administration of the Oligo VI abrogated the adenosine-induced bronchoconstriction in the three allergic rabbits. The actual efficacy of Oligo VI is, therefore, greater than could be measured in the experimental system used. By comparing with the previously submitted results for the Oligo I, it may be seen that the Oligo VI was found to be as effective, or more, than Oligo I.

Example 34: Conclusions

The work described and results discussed in the examples clearly indicates that all anti-sense oligonucleotides designed in accordance with the teachings of the above-identified application were found to be highly effective at countering or reducing effects mediated by the receptors they are targeted to. That is, each and all of the two anti-sense oligos targeting an adenosine A_1 receptor mRNA, 1 anti-sense oligo targeting an adenosine A_{2b} receptor mRNA, and the 2 anti-sense oligos targeting an A_3 receptor mRNA were shown capable of countering the effect of exogenously administered adenosine which is mediated by the specific receptor they are targeted to. The activity of the anti-sense oligos of this invention, moreover, is specific to the target and substitutively fails to inhibit another target. In addition, the results presented also show that the administration of the present agents results in extremely low or non-existent deleterious side effects or toxicity. This represents 100% success in providing agents that are highly effective and specific in the treatment of bronchoconstriction and/or inflammation. This invention is broadly applicable in the same manner to all gene(s) and corresponding mRNAs encoding proteins involved in or associated with airway diseases. A

comparison of the phosphodiester and a version of the same oligonucleotide wherein the phosphodiester bonds are substituted with phosphorothioate bonds evidenced an unexpected superiority for the phosphothiorate oligonucleotide over the phosphodiester anti-sense oligo.

Example 35: In Vivo Response to Adenosine Challenge with & without Oligo I Pretreatment

Two hyper responsive monkeys (ascaris sensitive) were challenged with inhaled adenosine, with and without pre-treatment with anti-sense oligo I (**SEQ.ID NO: 1**). The PC_{40} adenosine was calculated from the data collected as being equivalent to that amount of adenosine in mg that causes a 40% decrease in dynamic compliance in hyper-responsive airways. The Oligo I (**SEQ. ID NO:1**; EPI 2010) was subsequently administered at 10 mg/day for 2 days by inhalation. On the third day, the PC adenosine was again measured. The PC_{40} adenosine value prior to treatment with Oligo I was compared side-by-side with to the PC_{40} adenosine taken after administration of Oligo I (Figure not shown). The results of the experiment conducted with two animals showed that any sensitivity to adenosine was completely eliminated by the administration of the oligo of this invention in one animal, and substantially reduced in the second.

Example 36: Extension of the experimental Results

The method of the present invention is also practiced with anti-sense oligonucleotides targeted to many genes, mRNAs and their corresponding proteins as described above, in essentially the same manner as given above, for the treatment of various conditions in the lungs. Examples of these are Human A2a adenosine receptor, Human A2b adenosine receptor, Human IgE receptor β, Human Fc-epsilon receptor CD23 antigen (IgE receptor), Human IgE receptor, α subunit, Human IgE receptor, Fc epsilon R, Human histidine decarboxylase, Human beta tryptase, Human tryptase-I, Human prostaglandin D synthase, Human cyclooxygenase-2, Human eosinophil cationic protein, Human eosinophil derived neurotoxin, Human eosinophil peroxidase, Human intercellular adhesion molecule-1 (CAM-1), Human vascular cell adhesion molecule 1 (VCAM-1), Human endothelial leukocyte adhesion molecule (ELAM-1), Human P Selectin, Human endothelial monocyte activating factor, Human IL3, Human IL4, Human IL5, Human IL6, Human monocyte-derived neutrophil chemotactic factor, Human neutrophil elastase (medullasin), Human neutrophil oxidase factor, Human cathepsin G, Human defensin 1, Human defensin 3, Human macrophage inflammatory protein-1-alpha, Human muscarinic acetylcholine receptor HM1, Human muscarinic acetylcholine receptor HM3, Human fibronectin, Human interleukin 8, Human GM-CSF, Human tumor necrosis factor α, Human leukotriene C4 synthase, Human major basic protein, and many more.

The foregoing examples are illustrative of the present invention, and are not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

WHAT IS CLAIMED AS NOVEL & UNOBVIOUS IN UNITED STATES LETTERS PATENT IS:

1. A pharmaceutical composition, comprising

an oligonucleotide(s) (oligo(s)) which is (are) effective for alleviating bronchoconstriction and/or lung inflammation, allergy(ies), or surfactant depletion or hyposecretion, when administered to a mammal, the oligo containing about 0 to about 15% adenosine (A) and being anti-sense to a target selected from the group consisting of the initiation codon, the coding region, the 5'-end and the 3'-end genomic flanking regions, the 5' and 3' intron-exon junctions, and regions within 2 to 10 nucleotides of the junctions of a gene encoding a target polypeptide associated with lung airway dysfunction or anti-sense to the polypeptide mRNA; combinations of the oligos; and mixtures of the oligos; and

a pharmaceutically or veterinarily acceptable carrier or diluent.

2. The composition of claim 1, wherein the oligo is A-free.

3. The composition of claim 1, wherein the target is selected from the group consisting of the initiation codon, the coding region, the 5'-end and the 3'-end genomic flanking regions, the 5' and 3' intron-exon junctions, and regions within 2 to 10 nucleotides of the junctions of an oncogene(s) and a gene(s) encoding a target polypeptide(s) associated with lung airway dysfunction or anti-sense to the oncogene mRNA and the polypeptide mRNA; combinations of the oligos; and mixtures of the oligos; the polypeptides being selected from the group consisting of peptide factors and transmitters, antibodies, cytokines and chemokines, enzymes, binding proteins, adhesion molecules, their receptors, and malignancy associated proteins.

4. The composition of claim 3, wherein the target is selected from the group consisting of the initiation codon, the coding region, the 5'-end and the 3'-end genomic flanking regions, the 5' and 3' intron-exon junctions, and regions within 2 to 10 nucleotides of the junctions of an oncogene(s) and a gene(s) encoding a target polypeptide(s) associated with lung airway dysfunction or anti-sense to the oncogene mRNA and the polypeptide mRNA; combinations of the oligos; and mixtures of the oligos; wherein the polypeptides are selected from the group consisting of transcription factors, stimulating and activating peptide factors, cytokines, cytokine receptors, chemokines, chemokine receptors, adenosine receptors, bradykinin receptors, endogenously produced specific and non-specific enzymes, immunoglobulins and antibodies, antibody receptors, central nervous system (CNS) and peripheral nervous and non-nervous system receptors, CNS and peripheral nervous and non-nervous system peptide transmitters, adhesion molecules, defensins, growth factors, vasoactive peptides and receptors, binding proteins, and malignancy associated proteins.

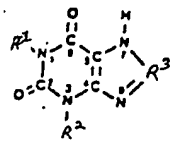
5. The agent of claim 4, wherein the encoded polypeptide(s) is(are) selected from the group consisting of adenosine receptors A1, A2a, A2b and A3, bradykinin receptors B1 and B2, Nf6B Transcription Factor, Interleukin-8 Receptor (IL-8 R), Interleukin 5 Receptor (IL-5 R), Interleukin 4 Receptor (IL-4 R), Interleukin 3 Receptor (IL-3 R), Interleukin-1β (IL-1β), Interleukin 1β Receptor (IL- 1β R), Eotaxin, Tryptase, Major Basic Protein, β2-adrenergic Receptor Kinase, Endothelin Receptor A, Endothelin Receptor B, Preproendothelin, Bradykinin B2 Receptor, IgE High Affinity Receptor, Interleukin 1 (IL-1), Interleukin 1 Receptor (IL-1 R), Interleukin 9 (IL-9), Interleukin-9 Receptor (IL-9 R), Interleukin 11 (IL-11), Interleukin-11 Receptor (IL-11 R), Inducible Nitric Oxide Synthase, Cyclo-oxygenase-1 (COX-1), Cyclo-oxygenase-2 (COX-2), Intracellular Adhesion Molecule 1 (ICAM-1) Vascular Cellular Adhesion Molecule (VCAM), Rantes, Endothelial Leukocyte Adhesion Molecule (ELAM-1), Monocyte Activating Factor, Neutrophil Chemotactic Factor, Neutrophil Elastase, Defensin 1, 2 and 3, Muscarinic Acetylcholine Receptors, Platelet Activating Factor, Tumor Necrosis Factor α, 5-lipoxygenase, Phosphodiesterase IV, Substance P, Substance P Receptor, Histamine Receptor, Chymase, CCR-1 CC Chemokine Receptor, CCR-2 CC Chemokine Receptor, CCR-3 CC Chemokine Receptor, CCR-4 CC Chemokine Receptor, CCR-5 CC Chemokine Receptor, Prostanoid Receptors, GATA-3 Transcription Factor, Neutrophil Adherence Receptor, MAP Kinase, Interleukin-9 (IL-9), NFAT Transcription Factors, STAT 4, MIP-1α, MCP-2, MCP-3, MCP-4, Cyclophillins, Phospholipase A2, Basic Fibroblast Growth Factor, Metalloproteinase, CSBP/p38 MAP

Kinase, Tryptose Receptor, PDG2, Interleukin-3 (IL-3), Interleukin-1β (IL-1β), Cyclosporin A-Binding Protein, FK5-Binding Protein, α4β1 Selectin, Fibronectin, α4β7 Selectin, Mad CAM-1, LFA-1 (CD11a/CD18), PECAM-1, LFA-1 Selectin, C3bi, PSGL-1, E-Selectin, P-Selectin, CD-34, L-Selectin, p150,95, Mac-1 (CD11b/CD18), Fucosyl transferase, VLA-4, CD-18/CD11a, CD11b/CD18, ICAM2 and ICAM3, C5a, CCR3 (Eotaxin Receptor), CCR1, CCR2, CCR4, CCR5, LTB-4, AP-1 Transcription Factor, Protein kinase C, Cysteinyl Leukotriene Receptor, Tachychinnen Receptors (tach R), I6B Kinase 1 & 2, STAT 6, c-mas and NF-Interleukin-6 (NF-IL-6).

6. The composition of claim 1, wherein one or more As is(are) substituted by a universal base selected from the group consisting of heteroaromatic bases which bind to a thymidine base but have antagonist activity and less than about 0.3 of the adenosine base agonist or antagonist activity at the adenosine A_1, A_{2a}, A_{2b} and A_3 receptors.

7. The composition of claim 6, wherein the heteroaromatic bases are selected from the group consisting of pyrimidines and purines, which may be substituted by O, halo, NH_2, SH, SO, SO_2, SO_3, COOH and branched and fused primary and secondary amino, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, alkoxy, alkenoxy, acyl, cycloacyl, arylacyl, alkynoxy, cycloalkoxy, aroyl, arylthio, arylsulfoxyl, halocycloalkyl, alkylcycloalkyl, alkenylcycloalkyl, alkynylcycloalkyl, haloaryl, alkylaryl, alkenylaryl, alkynylaryl, arylalkyl, arylalkenyl, arylalkynyl, arylcycloalkyl, which may be further substituted by O, halo, NH_2, primary, secondary and tertiary amine, SH, SO, SO_2, SO_3, cycloalkyl, heterocycloalkyl and heteroaryl.

8. The composition of claim 7, wherein the pyrimidines and purines are substituted at a position selected from the group consisting of positions 1, 2, 3, 4, 7, and 8, and the pyrimidines and purines are selected from the group consisting of theophylline, caffeine, dyphylline, etophylline, acephylline piperazine, bamifylline, enprofylline and xantine having the chemical formula



wherein R^1 and R^2 are independently H, alkyl, alkenyl or alkynyl and R^3 is H, aryl, dicycloalkyl, dicycloalkenyl, dicycloalkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, O-cycloalkyl, O-cycloalkenyl, O-cycloalkynyl, NH_2-alkylamino-ketoxyalkyloxy-aryl and mono and dialkylaminoalkyl-N-alkylamino-SO_2 aryl.

9. The composition of claim 8, wherein the universal base is selected from the group consisting of 3-nitropyrrole-2'-deoxynucleoside, 5-nitro-indole, 2-deoxyribosyl-(5-nitroindole), 2-deoxyribofuranosyl-(5-nitroindole), 2'-deoxyinosine, 2'-deoxynebularine, 6H, 8H-3,4-dihydropyrimido [4,5-c] oxazine-7-one or 2-amino-6-methoxyaminopurine.

10. The composition of claim 1, where one or more methylated cytocine(s) (^{m}C) is(are) substituted for a C in one or more CpG dinocleotide(s), if present in the oligo(s).

11. The composition of claim 1, wherein one or more mononucleotide(s) of the oligo(s) is(are) linked or modified by one or more methylphosphonate, 5'-N-carbamate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene(methyimino) (MMI), methoxymethyl (MOM), methoxyethyl (MOE), methyleneoxy (methylimino) (MOMI), 2'-O-methyl, phosphoramidate, C-5 substituted residues, or combinations thereof.

12. The composition of claim 11, wherein the mononucleotide residues are linked by phosphorothioate residues.

13. The composition of claim 1, wherein the anti-sense oligo comprises about 7 to about 60 mononucleotides.

14. The composition of claim 1, wherein the anti-sense oligo comprises fragments 1, 3,

5, 7 and 8 to 2313 (SEQ. ID NOS: 1 through 2419).

15. The composition of claim 1, wherein the anti-sense oligo is operatively linked to, or complexed with, an agent selected from the group consisting of cell internalized or up-taken agents and cell targeting agents.

16. The composition of claim 15, wherein the cell internalized or up-taken agent is selected from the group consisting of transferrin, asialoglycoprotein and streptavidin.

17. The composition of claim 1, wherein the oligo is operatively linked to a vector that is a prokaryotic or eukaryotic vector.

18. The composition of claim 1, wherein the oligo(s) is(are) hybridized to a ribonucleic acid.

19. A cell, carrying the oligo of claim 1.

20. The composition of claim 1, wherein the carrier or diluent is selected from the group consisting of gaseous, liquid, and solid carriers or diluents.

21. The composition of claim 20, further comprising an agent selected from the group consisting of other therapeutic agents, surfactants, flavoring and coloring agents, fillers, volatile oils, buffering agents, dispersants, RNA inactivating agents, anti-oxidants, flavoring agents, propellants and preservatives.

22. The composition of claim 21, comprising one or more oligo(s), a surfactant, and a carrier or diluent for the oligo and the surfactant.

23. The composition of claim 21, wherein the the agent is an RNA inactivating agent which comprises an enzyme, optionally an ribozyme.

24. The composition of claim 1, wherein the anti-sense oligo is present in an amount of about 0.01 to about 99.99 w/w of the composition.

25. The composition of claim 1, which is a systemic or topical formulation.

26. The formulation of claim 25, selected from the group consisting of oral, intrabuccal, intrapulmonary, rectal, intrauterine, intratunor, intracranial, nasal, intramuscular, subcutaneous, intravascular, intrathecal, inhalable, transdermal, intradermal, intracavitary, implantable, iontophoretic, ocular, vaginal, intraarticular, otical, intravenous, intramuscular, intraglandular, intraorgan, intralymphatic, implantable, slow release and enteric coating formulations.

27. The formulation of claim 26, which is an oral formulation, wherein the carrier is selected from the group consisting of solid and liquid carriers.

28. The oral formulation of claim 27, which is selected from the group consisting of a powder, dragees, tablets, capsules, sprays, aerosols, solutions, suspensions and emulsions, optionally oil-in-water and water-in-oil emulsions.

29. The formulation of claim 25, which is a topical formulation, wherein the carrier is selected from the group consisting of creams, gels, ointments, sprays, aerosols, patches, solutions, suspensions and emulsions.

30. The formulation of claim 26, which is an injectable formulation, wherein the carrier is selected from the group consisting of aqueous and alcoholic solutions and suspensions, oily solutions and suspensions and oil-in-water and water-in-oil emulsions.

31. The formulation of claim 26, which is a rectal formulation, optionally a suppository.

32. The formulation of claim 26, which is a transdermal formulation, wherein the carrier is selected from the group consisting of aqueous and alcoholic solutions, oily solutions and suspensions and oil-in-water and water-in-oil emulsions.

33. The transdermal formulation of claim 32, which is an iontophoretic transdermal formulation, wherein the carrier is selected from the group consisting of aqueous and alcoholic solutions, oily solutions and suspensions and oil-in-water and water-in-oil emulsions, and wherein the formulation further comprises a transdermal transport promoting agent.

34. The formulation of claim 26, which is provided in an implant, a capsule or a cartridge.

35. The composition of claim 20, wherein the carrier is selected from the group consisting of aqueous and alcoholic solutions and suspensions, oily solutions and suspensions and oil-in-water and water-in-oil emulsions.

36. The formulation of claim 20, wherein the carrier comprises a hydrophobic carrier.

37. The formulation of claim 36, wherein the carrier comprises lipid vesicles, optionally liposomes, or particles, optionally microcrystals.

38. The formulation of claim 37, wherein the carrier comprises liposomes, and the liposomes comprise the anti-sense oligo.

39. The formulation of claim 26, which is a respirable or inhalable formulation, optionally an aerosol.

40. The composition of claim 1, in single or multiple unit form.

41. The composition of claim 1, in bulk.

42. A kit, comprising

a delivery device;

in a separate container(s), the oligo(s) of claim 1; and

instructions for adding a carrier and for use of the kit.

43. The kit of claim 42, wherein the formulation is a respirable formulation and the delivery device comprises a nebulizer which delivers single metered doses of the formulation.

44. The kit of claim 43, wherein the nebulizer comprises an insufflator and the composition is provided in a piercable or openable capsule or cartridge.

45. The kit of claim 44, wherein the delivery device comprises a pressurized inhaler and the composition comprises a suspension, solution or dry formulation of the oligo.

46. The kit of claim 45, further comprising, in a separate container, an agent selected from the group consisting of other therapeutic agents, surfactants, anti-oxidants, flavoring agents, fillers, volatile oils, dispersants, antioxidants, propellants, preservatives, buffering agents, RNA inactivating agents, cell-internalized or up-taken agents and coloring agents.

47. The kit of claim 46, comprising, in separate containers, one or more oligos, one or more surfactants, and a carrier or diluent, and optionally other therapeutic agents.

48. The kit of claim 42, wherein the device is a transdermal delivery device, and the kit further comprises a transdermal delivery agent, a transdermal carrier or diluent, and instructions for preparing a transdermal delivery formulation.

49. The kit of claim 42, wherein the device is an iontophoretic delivery device, and the kit further comprises iontophoretic agents and instructions for preparing an iontophoretic formulation.

50. An in vivo method of delivering an anti-sense oligonucleotide(s) (oligo(s)) to one or more target polynucleotide(s), comprising administering into the respiratory system of a subject one or more oligo(s) that are anti-sense to the polynucleotide(s), in an amount effective to reach and hybridize to the target polynucleotide(s), and reduce the production or availability, or to increase the degradation, of the target mRNA, or to reduce the amount of the target polypeptide present in the lungs.

51. An in vivo method of delivering an anti-sense oligonucleotide (oligo) to a target polynucleotide associated with bronchoconstriction and/or lung inflammation, allergy(ies) and/or surfactant hypoproduction, comprising administering to a subject the composition of claim 1, that comprises an amount of the oligo(s) effective to reach and hybridize to the target polynucleotide(s), and reduce or inhibit the polynucleotide(s)' transcription and/or expression and, thereby, alleviating bronchoconstriction and/or lung inflammation, allergy(ies) and/or surfactant hypoproduction.

52. The method of claim 51, wherein the administered composition comprises an amount of the oligo(s) and is administered under conditions effective for alleviating bronchoconstriction and/or lung inflammation, allergy(ies) and/or surfactant depletion or hyposecretion, when administered to a mammal.

53. The method of claim 51, wherein the composition is administered into the subject's respiratory system.

54. The method of claim 53, wherein the composition is administered directly into the subject's lung (s).

55. The method of claim 51, wherein the administered composition comprises an amount of the oligo(s) and is administered under conditions effective to reduce the production or availability, or to increase the degradation, of the target mRNA or to reduce the amount of the target polypeptide present in the lungs.

56. The method of claim 51, wherein the agent is administered as a respirable aerosol.

57. The method of claim 51, wherein the pulmonary obstruction, and/or bronchoconstriction and\or lung inflammation, allergy(ies) and/or surfactant hypoproduction are associated with a disease or condition selected from the group consisting of pulmonary vasoconstriction, inflammation, allergies, asthma, impeded respiration, respiratory distress syndrome (RDS), pain, cystic fibrosis (CF), allergic rhynitis (AR), pulmonary hypertension, emphysema, chronic obstructive pulmonary disease (COPD), pulmonary transplantation rejection, pulmonary infections, bronchitis, and cancer.

58. The method of claim 57, wherein the disease or condition is associated with an allergy(ies), and the oligo is anti-sense to a target selected from the group consisting of the initiation codon,the coding region, the 5'-end and the 3'-end genomic flanking regions, the 5' and 3' intron-exon junctions, and regions within 2 to 10 nucleotides of the junctions of a gene(s) encoding an immunoglobulin(s) and antibody(ies) and immunoglobulin and antibody receptors or are anti-sense to the immunoglobulin(s) and antibody(ies) and immunoglobulin and antibody receptors mRNA; combinations of the oligo(s); and mixtures of the oligos.

59. The method of claim 57, wherein the disease or condition is associated with a malignancy or cancer, and the oligo is anti-sense to a target selected from the group consisting of the initiation codon,the coding region, the 5'-end and the 3'-end genomic flanking regions, the 5' and 3' intron-exon junctions, and regions within 2 to 10 nucleotides of the junctions of an oncogene(s) and\or encodes a malignancy associated protein, or is(are) anti-sense to the oncogene or malignancy associated protein mRNA; combinations of the oligo(s); and mixtures of the oligos and the oligo(s) is(are) administered in an amount effective to reduce either the level of the protein mRNA or of the malignancy associated protein, or to reduce the growth of or provide beneficial characteristics to malignant cells.

60. The method of claim 51, wherein the composition is administered transdermally or systemically.

61. The method of claim 60, wherein the composition is administered orally, intracavitarily, intranasally, intraanally, intravaginally, intrauterally, intraarticularly, transdermally, intrabucally, intravenously, subcutaneously, intramuscularly, intravascularly, intratumorously, intraglandularly, intraocularly, intracranial, into an organ, intravascularly, intrathecally, intralymphatically, intraotically, by implantation, by inhalation, intradermally, intrapulmonarily, intraotically, by slow release, by sustained release and by a pump.

62. The method of claim 51, wherein the subject is a non-human mammal.

63. The method of claim 51, wherein the mammal is a human.

64. The method of claim 51, wherein the oligo is administered in amount of about 0.005 to about 150 mg/kg body weight.

65. The method of claim 51, wherein the oligo is obtained by

(a) selecting fragments of a target nucleic acid having at least 4 contiguous nucleic acids selected from the group consisting of G and C;

(b) obtaining a first oligonucleotide 4 to 60 nucleotides long which comprises the selected fragment and has a C and G nucleic acid content of up to and including about 15%; and

(c) obtaining a second oligonucleotide 4 to 60 nucleotides long comprising a sequence which is anti-sense to the selected fragment, the second oligonucleotide having an A base content of up to and including about 15%.

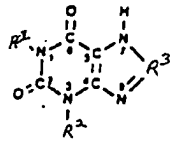
65. The method of claim 64, wherein the oligo is A-free.

66. The method of claim 51, wherein the target is selected from the group consisting of the initiation codon, the coding region, the 5'-end and the 3'-end genomic flanking regions, the 5' and 3' intron-exon junctions, and regions within 2 to 10 nucleotides of the junctions of an oncogene or a gene encoding a target polypeptide associated with lung airway dysfunction or anti-sense to the polypeptide or oncogene mRNA; combinations of the oligo(s); and mixtures of the oligos; wherein the polypeptide isselected from the group consisting of transcription factors, stimulating and activating factors, interleukins, interleukin receptors, chemokines, chemokine receptors, endogenously produced specific and non-specific enzymes, immunoglobulins, antibody receptors, central nervous system (CNS) and peripheral nervous and non-nervous system receptors, CNS and peripheral nervous and non-nervous system peptide transmitters, adhesion molecules, defensines, growth factors, vasoactive peptides, peptide receptors and binding proteins, and malignancy associated proteins.

67. The method of claim 51, wherein one or more As in the oligo(s) is(are) substituted by a universal base selected from the group consisting of heteroaromatic bases which bind to a thymidine base but have less than about 0.3 of the adenosine base agonist or antagonist activity at an adenosine A_1, A_{2a}, A_{2b} and A_3 receptors.

68. The method of claim 67, wherein the heteroaromatic bases are selected from the group consisting of pyrimidines and purines, which may be substituted by O, halo, NH_2, SH, SO, SO_2, SO_3, COOH and branched and fused primary and secondary amino, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, alkoxy, alkenoxy, acyl, cycloacyl, arylacyl, alkynoxy, cycloalkoxy, aroyl, arylthio, arylsulfoxyl, halocycloalkyl, alkylcycloalkyl, alkenylcycloalkyl, alkynylcycloalkyl, haloaryl, alkylaryl, alkenylaryl, alkynylaryl, arylalkyl, arylalkenyl, arylalkynyl, arylcycloalkyl, which may be further substituted by O, halo, NH_2, primary, secondary and tertiary amine, SH, SO, SO_2, SO_3, cycloalkyl, heterocycloalkyl and heteroaryl.

69. The method of claim 67, wherein the pyrimidines and purines are substituted at positions 1, 2, 3, 4, 7 and 8 and the pyrimidines and purines are selected from the group consisting of theophylline, caffeine, dyphylline, etophylline, acephylline piperazine, bamifylline, enprofylline and xantine having the chemical formula



wherein R^1 and R^2 are independently H, alkyl, alkenyl or alkynyl and R^3 is H, aryl, dicycloalkyl, dicycloalkenyl, dicycloalkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, O-cycloalkyl, O-cycloalkenyl, O-cycloalkynyl, NH_2-alkylamino-ketoxyalkyloxy-aryl and mono and dialkylaminoalkyl-N-alkylamino-SO_2 aryl.

70. The method of claim 69, wherein the universal base is selected from the group consisting of 3-nitropyrrole-2'-deoxynucleoside, 5-nitro-indole, 2-deoxyribosyl-(5-nitroindole), 2-deoxyribofuranosyl-(5-nitroindole), 2'-deoxyinosine, 2'-deoxynebularine, 6H, 8H-3,4-dihydropyrimido [4,5-c] oxazine-7-one or 2-amino-6-methoxyaminopurine.

71. The method of claim 51, further comprising substituting a methylated cytocine (^mC) for a C in one or more CpG dinucleotide(s), if present in the oligo(s).

72. The method of claim 51, further comprising substituting by, or modifying one or more nucleotide residue(s) of the oligo(s) with, methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene(methyimino) (MMI), methoxymethyl (MOM), methoxyethyl (MOE), methyleneoxy

(methylimino) (MOMI), methoxy methyl (MOM), 2'-O-methyl, phosphoramidate, C-5 substituted residues, or combinations thereof.

73. The method of claim 51, further comprising operatively linking to, or complexing the oligo(s) with, an agent selected from the group consisting of cell internalized and up-taken agent(s) and cell targeting agents.

74. The method of claim 73, wherein the cell internalized or up taken agent is selected from the group consisting of transferrin, asialoglycoprotein, and streptavidin.

75. The method of claim 73, wherein the cell targeting agent is a vector, optionally a prokaryotic or eukaryotic vector.

76. A method of treating a disease or condition associated with a target selected associated with a disease or condition afflicting lung airways, comprising conducting the method of claim 56.

77. The method of claim 76, wehrein the amount of oligo(s) administered is (are) effective to reduce the production or availability, or to increase the degradation, of the mRNA, or to reduce the amount of the polypeptide present in the lungs.

78. The method of claim 77, wherein the amount of oligo(s) administered is (are) effective to reduce the production or availability, or to increase the degradation, of the mRNA, or to increase the amount of the surfactant present in the subject's lungs.

79. The composition of claim 4, wherein the oligo(s) is(are) anti-sense to the initiation codon, the coding region, the 5'-end and the 3'-end genomic flanking regions, the 5' and 3' intron-exon junctions, and regions within 2 to 10 nucleotides of the junctions of a gene(s) encoding an adenosine A1, A2a, A2b and\or A3 receptor, or anti-sense to the adenosine A1, A2a, A2b and\or A3 receptor mRNA.

80. The composition of claim 79, wherein all nucleotide linking residues are phosphorothioates.

81. The composition of claim 1, wherein the oligo is a DNA.

82. The composition of claim 1, wherein the oligo is an RNA.

83. The composition of claim 1, wherein the oligo comprises about 7 to up to about 60 mononucleotides.

84. The composition of claim 79, wherein the oligo(s) is selected from the group consisting of fragment(s) SEQ ID NOS: 1, 3, 5, 7, 8, and\or 11 through 2419, optionally wherein at least one mononucleotide residue is substituted or modified by methylphosphonate, phosphotriester, phosphorothioate, phosphorodithioate, boranophosphate, formacetal, thioformacetal, thioether, carbonate, carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene(methyimino), (MMI), methoxymethyl (MOM), methoxyethyl (MOE), methyleneoxy (methylimino) (MOMA), methoxy methyl (MOM), 2'-O-methyl, phosphoramidate residues and/or combinations thereof.

85. The method of claim 51, wherein the oligo is administered topically to the airway, respiratory or pulmonary epithelium of the subject.

86. The composition of claim 1, wherein the oligo has a particle size of about 5-10 μm or in the range of 10-500 μm.

87. The composition of claim 1, further comprising a propellant.

88. The method of claim 50, wherein the oligo has a particle size of about 5-10 μm or in the range of 10-500 μm.

89. The method of claim 50, further comprising adding to the oligo a propellant.

90. The method of claim 51, wherein the oligo has a particle size of about 5-10 μm or in the range of 10-500 μm.

91. The method of claim 51, further comprising adding to the oligo a propellant.

SEQUENCE LISTING

(1) GENERAL INFORMATION
(i) APPLICANT: East Carolina University et al.
(ii) TITLE OF THE INVENTION: LOW ADENOSINE OLIGONUCLEOTIDE AGENT, COMPOSITION, KIT & TREATMENTS
(iii) NUMBER OF SEQUENCES: 3110
(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: ARTER & HADDEN
(B) STREET: 725 South Figueroa St, # 3400
(C) CITY: Los Angeles
(D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 90071
(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0
(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US99/
(B) FILING DATE: 3-AUG-1999
(C) CLASSIFICATION: UNKNOWN
(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/095,212
(B) FILING DATE: 03-AUG-1998
(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Amzel, Viviana
(B) REGISTRATION NUMBER: 30,930
(C) REFERENCE/DOCKET NUMBER: EPI-109
(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 213-430-3520
(B) TELEFAX: 213-617-9255
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
GATGGAGGGC GGCATGGCGG G 21

(2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
GTAGCAGGCG GGGATGGGGG C 21

(2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
GTTGTTGGGC ATCTTGCC 18

(2) INFORMATION FOR SEQ ID NO:4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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AGTGCCTACC CCAGCTC

(2) INFORMATION FOR SEQ ID NO:3004:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 209279 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3004:

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2) INFORMATION FOR SEQ ID NO:3005:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11786 base pairs
(B) TYPE: nucleic acid